Electroplating wastewater polishing in constructed wetland systems

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ELECTROPLATING WASTEWATER POLISHING IN CONSTRUCTED WETLAND SYSTEMS

EFFICACITE DES ZONES HUMIDES CONSTRUITES POUR LE POLISSAGE DES EAUX USEES EN PROVENANCE DE L'INDUSTRIE DE LA GALVANOPLASTIE

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Doctoral thesis

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List of abbreviations and acronyms

A	volume of wetland containing water in active flow
ABG	above-ground
b	sorption energy
BCF	bioconcentration factor
BCR	Community Bureau of Reference
BLG	below-ground
bot	bottom
BSE	backscattered electron (detector)
C_e	equilibrium concentration in the solution
C_{in}	influent concentration of contaminant
COD	chemical oxygen demand
C_{out}	effluent concentration of contaminant
CRM	certified reference material
CW	constructed wetland
d	diameter
DF	downflow
DW	dry weight
ε	porosity
EDS	energy-dispersive X-ray spectroscopy
FAAS	flame atomic absorption spectroscopy
FaD	fill-and-drain
FaD-C	fill-and-drain (system) with compost
FaD-CP	vegetated fill-and-drain (system) with compost
FaD-CU	unvegetated fill-and-drain (system) with compost
FaD-G	fill-and-drain (system) with gravel
FaD-GP	vegetated fill-and-drain (system) with gravel
FaD-PP	vegetated fill-and-drain (system) with peat
FaD-S	fill-and-drain (system) with sand
FaD-SP	vegetated fill-and-drain (system) with sand
FWS	free water surface
h	water depth
H	height
HF	horizontal flow
HRT	hydraulic retention time
ICP-AES	inductively coupled plasma – atomic emission spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
k	areal rate constant
K_p	dissociation constant
K_s	solubility product constant
MAD	median absolute deviation
ORP	oxidation-reduction potential
pK	negative common logarithm of K_p
q	hydraulic loading rate
Q	inlet flow rate
q_s	concentration adsorbed
R_A	area-adjusted removal rate
SD	standard deviation
SEM	scanning electron microscopy
SEP	sequential extraction procedure

SRB	sulphate-reducing bacteria
TF	translocation factor
TOC	total organic carbon
TopS	top (layer) of substrate
UF	upflow
UF-C	upflow (system) with compost
UF-CP	vegetated upflow (system) with compost
UF-CU	unvegetated upflow (system) with compost
UF-G	upflow (system) with gravel
UF-GP	vegetated upflow (system) with gravel
UF-GU	unvegetated upflow (system) with gravel
UF-P	upflow (system) with peat
UF-PP	vegetated upflow (system) with peat
UF-PU	unvegetated upflow (system) with peat
UF-S	upflow (system) with sand
UF-SP	vegetated upflow (system) with sand
UF-SU	unvegetated upflow (system) with sand
WWTP	wastewater treatment plant
X_m	Langmuir adsorption maximum

1 Introduction

It is believed that environmental pollution caused by metals began with domestication of fire. Small amounts of trace metals released while burning of firewood altered metal levels in the domesticators' closest environment, a cave. Based on various natural deposits it was discovered that significant emission of metals to the environment occurred during the time of Roman Empire followed by mining and smelting industry renaissance in Europe and then the Industrial Revolution (Nriagu, 1996). The most common practice over the centuries of metals resources exploitation was to mine, to use and then to discard without being aware of the environmental impact (Odum et al., 2000).

Electroplating industries generate a large volume of wastewater, which contains high amounts of metals and cyanides. Thus, electroplating wastewater may cause a serious threat to the environment if not treated properly (Qin et al., 2002). Electroplating wastewater treatment or polishing in constructed wetlands (CWs) was studied to a very limited degree. This disallows any conclusions regarding broader application of CWs as a polishing step added to conventional treatment systems. Noteworthy, CWs were receiving a great deal of interest as a viable solution to acid mine drainage mitigation (Kadlec and Wallace, 2009). Presumably, electroplating wastewater having some traits of acid mine drainage should be treated in CWs with similar efficiency. It is, however, merely a hypothesis, which should be studied. High concentrations of toxic pollutants in the electroplating effluents preclude direct application of biological methods for their treatment. Thus, CWs are not an alternative to conventional physical-chemical methods of electroplating wastewater treatment. However, CWs may serve as a polishing step following conventional treatment of electroplating wastewater prior to its discharge into receiving waters or sewer but also as a buffer volume in case of failure of the upstream treatment facilities. In the long-term perspective they can remove the marginal concentrations of heavy metals that cannot be removed by classical physical-chemical methods (Diels et al., 2003).

The concept of using CWs for cost- and energy-efficient treatment of industrial wastewaters in the world has been demonstrated with a high degree of success (exhaustive overview is provided by Kadlec and Wallace (2009)). Wetlands have a high potential to remove metals and cyanides, but highly contaminated substrate would need to be regarded as a hazardous material for final disposal after a period which can span from a few to hundred years (Eger and Lapakko, 1989; Kadlec and Wallace, 2009; Gessner et al., 2005). The removal of metals in subsurface flow CWs is dominated by four mechanisms: adsorption, precipitation of metal sulphides or (oxy)hydroxides, and deposition of suspended solids. It should be noted that a minor fraction of

metals is removed by hydrophytes, which unlike terrestrial plants, cannot hyperaccumulate metals (Marchand et al., 2010). The major advantages of using CWs for the treatment of industrial wastewater as compared to active chemical treatment are: (i) that they are more cost-effective, particularly in the long-term operation, (ii) that they employ natural biological processes; and (iii) that they do not produce bulky oxidized sludges that require further management (Johnson and Hallberg, 2002). The term ‘passive systems’ has been often used in reference to CWs treating industrial wastewater, however, not all of them can be termed so. There are several types of active-mode CWs that allow for better control of the treatment process but require more energy and maintenance. Two types of these CWs and their application to electroplating effluent will be discussed in detail in this thesis.

2 Goals of the thesis

The use of CWs for the treatment of wastewater containing metals and cyanide has been reported in the literature, however the understanding of the processes responsible for reduction of these contaminants can be still improved. This is especially important to emphasize the scarcity of data regarding treatment of electroplating wastewater in CWs. It may be hypothesized that CWs have a great potential for efficient polishing of electroplating wastewater. This can be based on the results for similar types of wastewater. These similarities may be somewhat misleading as wastewater is a complex matrix and the applicability of CWs cannot be based on a desk-study comparison without experimental confirmation. Also the electroplating wastewater may differ significantly regarding the composition, thus a single study would not be conclusive for all the electroplating effluents. The term 'polishing' is understood as a treatment of wastewater, pretreated by conventional methods, to the level that allows discharge into sewer or the environment. Thus, the polishing process would be employed to reduce residual amounts of metals and cyanides, which are invariably present in the electroplating wastewater.

The goals of the experiment described in this thesis were to: (i) study the feasibility of polishing electroplating effluent in CW systems, (ii) study the effect of operating schemes, plants, bed media, wastewater strength and carbon source, (iii) to elucidate metal removal mechanisms in CWs.

This experiment may contribute to both applied and fundamental sciences. The first two goals were set to gain new knowledge on the application of CWs for the polishing of electroplating wastewater. This is, based on the previous experiences reported in the literature, for different types of wastewater containing metals and cyanides, it is reasonable to hypothesize that this type of wastewater could be efficiently treated in CWs. Additionally, the acceptable efficiency can be hypothetically achieved only when favourable conditions would prevail in the system. The evaluation of the system cannot be based merely on the comparison between influent and effluent data. The mechanisms responsible for the removal of contaminants cannot be regarded as an aspect belonging only to the domain of fundamental science. The knowledge of the major removal mechanisms allows prediction of the future performance of a system and its impact on the environment after its operation is terminated. Importantly, the information furnished by the small-scale studies could enhance the design of large-scale system by emphasizing those features of a system that promote the dominance of the most advantageous mechanism of removal.

The goals of the study were taken into account during the design of the experimental systems and in the experiment itself. In this study the applicability of CWs to polishing electroplating wastewater was tested both on real and simulated wastewater. This thesis presents the research carried out to meet these goals and the relevant results demonstrating key aspects of polishing electroplating wastewater in CWs.

3 Origin and treatment of electroplating wastewater

Electroplating is a process of applying metal coating to an object to enhance its protective properties and alter its appearance, or both. Metal coating is a step-wise process consisting of: degreasing, mechanical pretreatment, etching and, finally, electroplating in bath solutions. Between each step objects are rinsed to remove contamination and the residual bath solutions (Bartkiewicz, 2006).

3.1 Types of electroplating wastewater and the related contaminants

The principle types of the electroplating wastewater are bath solutions and rinse waters. Rinsing is a major contribution to the electroplating wastewater and is proportional to the electroplating plant's capacity. Rinse waters are continuously produced and contain relatively low concentrations of contaminants. Bath solutions, in contrast, contain markedly higher concentrations of metals and cyanides (on the order hundreds of g/L) and are exchanged every several weeks or months, depending on the process (Bartkiewicz, 2006). The electroplating wastewater contains chiefly residual metals, cyanides and solvents. Because of its toxicity electroplating effluent belongs to a category of waste streams, which are one of the most cumbersome in treatment and acute to the environment. It is a common practice to treat (to various extent) electroplating wastewater before its discharge into sewer system by the physical-chemical processes including chemical precipitation, electrolysis, reverse osmosis, and ion exchange (U.S. EPA, 1998). Metal precipitation as hydroxides has been the most favoured treatment option. Precipitation, by adjusting pH values, is not selective process and any iron present in the wastewater will precipitate first followed by other metals. The important drawback of hydroxide precipitation is that it produces large quantities of solid sludge for disposal (Eccles, 1995). It is noteworthy that conventional primary and secondary unit processes at municipal wastewater treatment plants (WWTPs) are not fully adequate for efficient removal of heavy metals and cyanides of electroplating origin, and the biological processes may be inhibited by these contaminants if the biomass was not previously adapted (Torrens, 2000; Henriques et al., 2007). In particular, nitratation was reported to be inhibited at concentration in excess of 1 mg/L of free cyanide (Kim et al., 2011). Moreover, treatment of wastewater containing metals by activated sludge impairs the quality of the excess sludge precluding its agricultural application (Chipasa, 2003). Thus, even the polishing of wastewater discharged into municipal sewer should be important from the environmental perspective. The other approach might be to polish the effluent of municipal wastewater treatment plants (especially small- and medium-sized systems) treating wastewater with significant fraction of industrial effluents (Ranieri and Young, 2012).

3.2 Legal regulations for the quality of electroplating wastewater

The quality regulations for electroplating wastewater and other industrial effluents have been set by the Regulation (Dz.U. 2006 nr 137 poz. 984) of the Polish Ministry of Environment (PL MoE, 2006). The maximum allowable concentration of the substances of concern in the research described in this thesis are given in Tab. 3.1. The CWs are not separately considered in this act of legislation but it should be mentioned that the sample filtration is required for the wastewater treated by biological ponds ('stawy biologiczne' in Polish). It was not, however, specified whether CWs or at least some of their variations can be regarded as biological ponds.

Table 3.1 Legal regulations for the quality of treated industrial wastewater (of selected substances) (excerpted from PL MoE, 2006)

Contaminant	**MAC[1], mg/L**
Aluminium	3
Boron	1
Free cyanide	0.1
Fixed cyanide	5
Copper	0.5
Iron	10
Nickel	0.5
Lead	0.5
Sulphate	500
Zinc	2
pH value	6.5−9.0

[1]maximum admissible concentration (or pH value) imposed by the Polish Ministry of Environment (PL MoE, 2006)

3.3 Treatment of electroplating wastewater: case study of the PEM Company

The PEM (subsidiary of SOFIPEM) electroplating plant in Siaugues-Sainte-Marie (Auvergne, France) specializes in electrolytic reel to reel plating process for many types of application. Some of the processes that are being conducted by the PEM are: application of electronic or electric connections, anodisation, brass plating, and colouring titanium by electrochemical oxidation. The noteworthy feature of the company is its flexibility in terms of service, depending on the customer requirements. The application areas of the products processed by PEM are: ski manufacturing, electromagnetic equipment industry, rail, space industry, medicine, watches, tires, aviation, household appliances, information technology, telecommunications industries, military industry, mechatronics, batteries and others. The metals which are being deposited on plated surface are: copper, tin, nickel, gold, silver and palladium. The substrates for deposition of the above-mentioned metals are: aluminium (and its alloys), ferronickel, steel and stainless steel, copper, brass, bronze, zinc (and its alloys), titanium (and its alloys). Some of these metals are present in the wastewater generated by the PEM, as water is used mostly in the plating shops

for rinsing of plated surfaces and as a plating bath. The other contaminant present in the electroplating wastewater of the PEM are cyanide compounds, which are used especially for copper and brass plating. The wastewater laden with cyanides is alkaline and contains also metal salts. Apart from metals and cyanides, electroplating wastewater contains also auxiliary compounds or elements such as: boron, fluoride, sulphates, nitrates, phosphates, surfactants and oils. Not all of these compounds were present or determined in the PEM wastewater, which will be presented and discussed further in this thesis (www.2). The WWTP in the PEM electroplating plant has the capacity of 30 m^3/h (www.2). The general layout of the WWTP is presented in Fig. 3.1. This schematic shows also the hypothetical CW system, which will be discussed in more detail in this thesis. This WWTP collects and then treats two streams of wastewater: alkaline cyanide wastewater and acidic wastewater, which both contain high concentration of metals. The two main treatment processes are intended to remove cyanides and metals form the wastewater. Prior to decyanidation process both streams are separated and then are blended and undergo metal precipitation process.

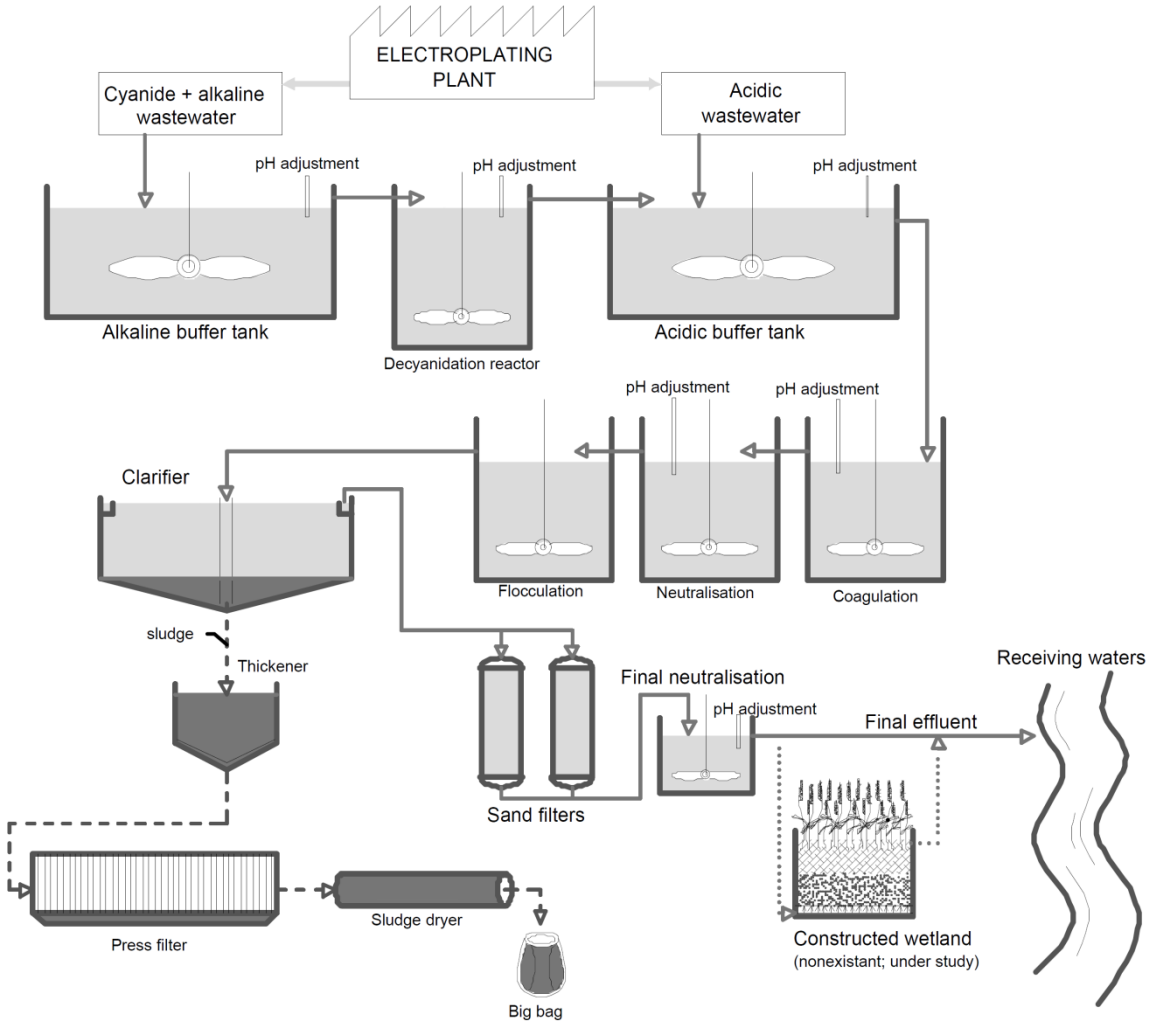


Figure 3.1 Layout of the WWTP at the PEM electroplating plant in Siaugues-Sainte-Marie (France)

Decyanidation of wastewater is the most commonly carried out using chlorine. The oxidant used in most cases is sodium hypchlorite (NaClO) at pH 10, which is advantageous for the second stage of the process when cyanogen chloride (CNCl) hydrolyzes to the cyanate ion (CNO^-). This ion can be oxidized further with chlorine at a nearly neutral pH to CO_2 and N_2. The main drawback of this method is that it entails significant increase of wastewater salinity: 5 mg of Cl^- per 1 mg of oxidized cyanide. The adverse effect of salinity increase using chlorine as an oxidizing agent can be avoided when hydrogen peroxide (H_2O_2) is used instead, which was employed at the PEM. The reaction of cyanide compounds with H_2O_2 occurs at fast rate and is believed not to mediate production of toxic by-produts (Eaton et al., 2004; Bartkiewicz, 2006); it occurs as follows:

$$CN^- + H_2O_2 = CNO^- + H_2O \quad (3.1)$$

The additional advantage of using H_2O_2 for oxidation of cyanide compounds is that it can be used in a broad range of pH, from 3 to 12. It is optimal at pH 9.5 and is catalysed by the presence of Cu^{2+} (Szafnicki et al., 2006).

Metal precipitation process takes place after acidic wastewater and treated cyanide wastewater have been blended. This process involves addition of alkali (NaOH) to increase the pH of wastewater up to the value when metal hydroxides have the lowest solubility:

$$Me^{+n} + nOH^- \rightarrow Me(OH)_n \quad (3.2)$$

The optimum pH of this process is between 9–10 at the PEM, as at this pH the hydroxide solubility of target metals Zn, Ni and Cu is very low. It should be mentioned that the pH value when the solubility of metal hydroxide is the lowest differs depending on metal and for this reason precipitation may occur in a step-wise manner with various pH (Szafnicki et al., 2005; Lewis, 2010). Coagulant and flocculating agents are also added to improve the process. Precipitates are separated from the wastewater in clarifiers and sand filters and then dried and the processed to recover metals by a specialized company. The pH of the final effluent is adjusted with sulphuric acid and discharged into a stream.

4 Constructed wetlands for the treatment of wastewater

The literature contains numerous terms used interchangeably to describe treatment wetlands and their types and modifications. The abundance of names follows from various perspectives, local nomenclatures, somewhat subjective selection of a trait that best describes a given system, and even the need of the author to add an air of innovation or novelty to their design. This diversity and inconsistency in nomenclature within the CW field may lead to confusion on various level of communication e.g. visible while browsing scientific databases (Fonder and Headley, 2011).

4.1 Classification of constructed wetlands

The nomenclature in this thesis will be used according to the terminology system proposed and discussed by Fonder and Headley (2011). The only exception from that scheme will be naming engineered wetland itself, here always referred to as 'constructed wetland' (CW) instead of 'treatment wetland', and defined as: "engineered system designed and constructed to utilize the natural processes involving wetland vegetation, soils, and their associated microbial community to assist in treating wastewater" according to Vymazal and Kröpfelova (2008). These two terms have been used interchangeably in literature to designate the same object. 'Constructed wetland', however, is a broader term than 'treatment wetland', as its designation can be used not only for treatment but also for other purposes including restoration, recreation and wildlife habitat (Fonder and Headley, 2011).

The three standard types of CWs with surface flow are (Fonder and Headley, 2011):

- Surface Flow (SF) CW, dominated by emergent herbaceous macrophytes;
- Free-Floating Macrophyte (FFM) CW containing free-floating vascular aquatic plants growing on the water surface.
- Floating Emergent Macrophyte (FEM) CW with emergent hydrophytes growing on a buoyant structure.

The four standard types with subsurface flow are:

- Horizontal Flow (HF) CW;
- Fill and Drain (FaD) CW in which the flow direction is mixed, normally alternating between up and down flow;
- Down Flow (DF) CW, which is free-draining and without surface flooding;
- Up Flow (UF) CW with a flooded surface.

The types of CWs can be also differentiated based on the needed energy input and possible performance control. In this respect two types of CWs can be distinguished. Type A systems are more similar to natural wetlands and they require low-energy input. Type B wetlands demand high-energy input and are more highly engineered systems. Type B CWs are in general more expensive in operation than Type A systems. Type B systems often require less footprint thus their construction does not always entail higher expenditures. In general, UF and FaD systems, being of interest in this thesis, can be classified as Type B systems as they require active management, as compared to passive mode systems, e.g. SF-CWs. Also the amendments of the system operation by chemical additions are typical for Type B systems (Kadlec and Wallace, 2009).

4.2 Subsurface flow CWs with vertical flow

Vertical subsurface CWs generally consist of a bed with porous media (sand or gravel) through which water flows in vertical direction. All CWs with vertical flow fall in the category of subsurface flow CWs. For this reason, the term ‘subsurface’ is redundant and will be omitted in this thesis. Noteworthy, vegetation type of vertical flow CWs is always emergent and does not play a role in classification or naming of these systems. In terms of flow direction CWs with vertical flow are subdivided into systems with downflow (DF), upflow (UF) and mixed flow with upward and downward direction (fill and drain, FaD) (Fonder and Headley, 2011). For brevity and to avoid confusion the term ‘vertical’ will be omitted in most cases in this thesis when naming systems with this flow direction.

DF CW is the most commonly applied type of vertical flow CW, which are free-draining and remain unsaturated for most of the time (Fonder and Headley, 2011). This type of CWs is the earliest form of vertical flow CWs and was initiated by dr Kathe Seidel in the early 1960s as a part of the Max Planck Institute Process (Kadlec and Wallace, 2009). The wastewater is distributed across the surface of the bed by a network of pipes in a manner that avoids surface flooding.

UF CWs are characterized by the fact that their bed is constantly saturated and permanently flooded over the surface. The term ‘saturated’ does not have to be a part of the name of this system as the upward flow implies clearly that the media remains saturated during normal operation of the system. In large-scale systems wastewater is introduced at the bottom of the bed by a series of distribution pipes and flow upwards to the surface. The surface of these systems is flooded for practical reason because it facilitates to convey the wastewater to the outlet. This

water layer may limit the oxygen transport into the bed. UF CWs are mostly applied when anaerobic treatment processes should be promoted especially for the treatment of mine waters and industrial wastewaters. The advantage of these CWs is that the clogging is smaller than in the HF CWs as the influent is distributed over a much larger area (Fonder and Headley, 2011).

FaD CWs have been classified as having mixed flow direction: upward and downward. The bed of these systems is intermittently saturated, which is related to the fill and drain phases. The common practice is that the surface of FaD CWs is not flooded. FaD CWs are mostly applied when increased oxygen transfer is needed (Fonder and Headley, 2011).

4.3 Use of constructed wetlands for industrial wastewater treatment

Constructed wetlands have been used to treat a variety of industrial wastewater including landfill leachate, dairy wastewater, acid mine drainage, winery wastes, deicing fluids, and others (Wallace and Knight, 2006; Obarska-Pempkowiak et al., 2010). The application of CWs for the treatment of electroplating wastewater was reported scarcely in the literature. Ranieri and Young (2012) studied the removal of Ni and Cr (and other contaminants) from municipal wastewater containing about 20% of electroplating and textile wastewater. This study consisted in monitoring of a full-scale CW with HF. The treatment of electroplating effluents was also investigated in the system with water hyacinth in Asia (Yeoh, 1983; Abbasi, 1987). Vymazal and Kröpfelová (2008) referred to the work of Shroff (1982) that was dealing to some of extent with the treatment of electroplating wastewater. On the Web Of Knowledge (www.3) a query including ‘electroplating’ and ‘wetland*’ (as topics) yielded only 6 results (as of January 2013). Five of which dealt with interactions between plants and metals, and one regarding mobility of chromium in natural wetlands. Noteworthy, none of these articles reported application of CWs for the treatment of electroplating wastewater. Although the treatment of electroplating wastewater in CW have not so far received much attention, there are numerous examples of other metal-laden wastewater that were discharged into CWs and treated with various efficiency. These types of wastewater are in particular: mine water, tannery wastewater, and landfill leachate (Kadlec and Wallace, 2009). Especially, the literature reports widely on various aspects of treating acid mine drainage in CWs (PIRAMID, 2003; Lesage, 2006; Kadlec and Wallace, 2009). The main characteristics of acid mine drainage are low pH and high concentrations of dissolved metals (mainly iron, manganese and aluminium) and sulphates (Neculita et al., 2007). Despite many implementations of CWs for abatement of acid mine drainage the operational know-how is somewhat rudimentary, which often lead to failures of these systems (Kadlec and Wallace, 2009). Johnson and Hallberg (2002) stated that CWs commonly used for treating this

type of wastewater allow only little or even no control are subjected to seasonal variations. Moreover, the available data do not suffice for sound assessment of sizing and performance of this passive CWs (Type A systems). The improved control over the processes occurring in the CWs can be achieved in the Type B wetlands, e.g. in the UF or FaD systems. Some of these solutions were tested on bench-scale, however, no full-scale sizing guidelines exist for these types of CWs. It should be, however, mentioned that the PIRAMID Guidelines (2003) offer detailed information on sizing and selection of passive treatment (among others CWs) for acid mine drainage.

4.4 Intensification of treatment processes in constructed wetlands

The performance of CWs is limited by many factors. It is feasible to improve it by modifications to their construction or operational schemes. In the case of CWs used for the removal of cyanides and metals both construction and operation can improve the treatment efficiency. The removal of cyanides can be enhanced, for example, by adding grained iron oxide to complex cyanides and by applying surface flow system to enable photolysis (Álvarez, 2006). The reduction of metals is mostly dependent on the dominant removal mechanism, which should be stimulated to improve the performance of a system. The bacterial sulphate reduction can play a major role in the removal of metals and therefore the activity of responsible group of bacteria is a factor of concern. The strategies for stimulation of bacterial sulphate reduction are discussed in Section 5.4.3.

4.5 Physical models of constructed wetlands

Constructed wetlands are outdoor large-scale system affected by weather changes and wildlife. As the science has been using models for the investigated phenomena also CWs have been modelled, mathematically and physically. These physical models are often small-scale systems in pots, buckets, columns, tanks and others. The results of the small-scale studies cannot be directly translated into full-scale systems but might be useful in understanding how large CWs operate (Kadlec and Wallace, 2009). There are two terms ‘microcosm’ and ‘mesocosm’ which have been widely used in the literature interchangeably and confusingly to denote small-scale physical models of CWs. The two definitions of these experimental systems are following: “The size of mesocosms is somewhat arbitrary but, within the spectrum of experimental ecology, mesocosms fall between small-scale microcosms (such as contained in laboratory beakers) and whole, natural ecosystems (such as paired lakes or watersheds). They must be large enough to contain a fairly complex ecosystem but small enough to be constructed and manipulated for

experiments. Mesocosms are usually within the range of 1-10 m diameter or length and typical examples are artificial ponds, tanks, or raceways for aquatic systems, and large pots, or greenhouses for terrestrial systems" (Kangas and Addey, 2008). "Microcosms are similar to mesocosms but on a smaller scale. There are no strict definitions to delineate microcosms from mesocosms. However, microcosms are often considered to be structures of laboratory-bench scale while mesocosms are room size or larger" (Matheson, 2008).

Experimental microcosms are advantageous tools in science as they offer basic-science insights, allow for rapid results, can be less expensive than studies of large-scale systems, and allow for replication and thereby sound statistical analysis. The size and duration of microcosm studies may, however, exclude or distort important features of communities and ecosystems. Relatively brief experiments can be misleading because of inability to account for transient dynamics, indirect effects, environmental variability, multiple stable equilibria, and site history. On the other hand, microcosms can be useful to eliminate hypothesized mechanism, compare alternative mechanisms, or estimate rates. Hence, the microcosm CWs can be a valuable tool, but the results of the microcosm studies should be evaluated, if possible, on larger scale, and to avoid overintepretation (Carpenter, 1996).

5 Electroplating-related pollutants and their removal in constructed wetlands

5.1 Metals and metalloids

5.1.1 Occurrence in electroplating wastewater

The occurrence of heavy metals in industrial wastewater is a matter of concern because these contaminants are often present at significant levels and if discharged directly into the environment can have severe effects on the environment itself and public health. Metals at toxic levels may inhibit biological processes at the activated sludge systems (Kiptoo et al., 2004). The composition of electroplating wastewater depends on the type of coatings and objects used in the electroplating workshops. According to Bartkiewicz (2006) the most common decorative coating is copper-nickel-chromium, thus these metals are expected to occur in the electroplating wastewater in increased concentrations. Chromium, however, will not be investigated in this thesis because it was not used by the PEM Company (SOFIPEM, 2009).

5.1.2 Forms of metals in constructed wetlands

There are at least two definitions of chemical speciation. It is either "the active process of identification and quantification of the different species forms of phases in which an element occurs in a material" or "the description of the amounts and kinds of species, forms or phases present in the material" as proposed by Ure (1991). The same author suggested that speciation be divided into three classes:

- classical, which refers to specific chemical compounds or oxidation states;
- functional, which refers to the specific behaviour of the element in a particular environment or in specific conditions, and is characterized as 'plant available' or 'mobile' species;
- operational, which refers to the situation where the reagent used to extract the sample defines the species, e.g. 'acetic acid soluble' species.

Direct determination of the chemical form of metal in solid-phase environmental samples can be achieved by means of various instrumental techniques, such as synchrotron based X-ray radiation fluorescence, particle induced X-ray emission, X-ray adsorption near edge structure, and extended X-ray absorption fine structure spectroscopy (D'Amore et al., 2005; Bacon and Davidson, 2008). Only few methods are directly applicable to the mineralogical analysis of wetland bed media due to the poor crystallinity of the precipitates and poor detection limits

(Neculita et al., 2008). These methods include, scanning electron microscopy equipped for backscattered electron imaging (SEM-BSE), which was the most reliable tool, whereas X-ray diffraction (XRD) or iron Mossbauer analyses have been of lesser applicability in detecting amorphous metal sulphides (Machemer et al., 1993). The SEM approach coupled with X-ray microanalysis proved successful for identifying sulphides in reactive mixtures from CWs (Machemer et al., 1993; Song, 2003). Neculita et al. (2008) used the scanning electron microscopy - energy-dispersive X-ray spectroscopy (SEM-EDS) and XRD techniques to evaluate mineralogy of spent reactive mixtures withdrawn from bench-scale sulphate-reducing column reactors. Gammons and Frandsen (2001) examined the solids from anaerobic CWs using SEM and XRD.

The distribution, mobility and bioavailability of metals in the environment depend on their concentration and also (to even more extent) on the form in which they are bound to solid phase. The short-term or long-term changes of the chemical-physical conditions in the environment may increase the mobility of metals causing contamination. The trait of being bioavailable that is characteristic for metals is associated with many variables such as characteristics of the particle surface, on the type of binding, and properties of the solution in contact with the solid phase (Filgueiras et al., 2002). Another crucial factor deciding whether an element will be incorporated into an organism is its physiology. As stated by Bacon and Davidson (2008) functional speciation depends strongly on the context and aim of the experiment so that the same metal pool could be considered as “bioavailable” when plant uptake is of interest but “mobile” or “labile” in leaching studies.

The complexity of possible reactions and often unknown reaction kinetics in natural soils and sediments restrict studies of metal species distribution in solid phases mainly to operationally defined analytical procedures such as sequential extraction procedures (Tack and Verloo, 1995).

Sequential extraction procedures and their applicability to wetland substrates

Sequential extraction procedure (SEP) consists in application of a series of reagents added to the same sample to sub-divide the total metal content. Each step of the procedure tends to be more vigorous then the previous one, starting with initial mild conditions (e.g. shaking with water, a salt solution or dilute acetic acid) to end with much harsher reagents (e.g. hot mineral acid). Generally, the potentially toxic elements extracted early during the procedure are those most weakly bound to the solid phase. They have thus greater potential mobility and environmental impact than those extracted later. Large number of different protocols has been reported,

however, the Tessier (Tessier et al., 1979) and BCR (Quevauviller et al., 1994; Rauret et al., 1999) schemes are among the most common schemes (Bacon and Davidson, 2008). In an IUPAC report Hlavay et al. (2004) stated: "Despite some drawbacks, the sequential extraction method can provide a valuable tool to distinguishing among trace element fractions of different solubility related to mineralogical phases. The understanding of the speciation of trace elements in solid samples is still rather unsatisfactory because the appropriate techniques are operationally defined". This means that sequential extraction only divides the potentially toxic element content of a sample into portions soluble in particular reagents used under particular conditions. These reagents are often recommended in a given protocol to target well-defined mineral phases, but their specificity cannot be guaranteed in all cases. Thus, the results of only sequential extraction are insufficient to elucidate binding characteristics of trace metals to specific minerals. Reliable interpretation can be made only if additional analytical techniques are employed (e.g. X-ray diffraction or SEM-EDS) to analyse the residues after each stage of the extraction. It is understandable that the application of sequential extraction procedure is not advisable for absolute studies, this is, those in which distribution of metals between specific soil phases is to be identified (Bacon and Davidson, 2008).

The considerable advantage of SEPs is their ease of use, and that they can be adjusted to a wide variety of environmental samples. However, SEPs have significant limitations that may compromise their validity and that have to be borne in mind while performing and interpreting the results of the SEP. The most important of these limitations is the potential introduction of artifacts that obscure the true metal speciation (Tipping et al., 1985). These artifacts may be introduced by two routes: (i) redistribution of leached metals into other sediments phases, or (ii) partial or complete dissolution of sediment phases prior to their targeted extraction step. In the first case this artifactual extra content of metals will be quantified in the following fractions, whereas in the second case later fractions (e.g. reducible) will be underestimated (Peltier et al., 2005). The latter problem becomes particularly important when anoxic sediments are being studied by SEP. It regards samples taken from various environments, but natural and CWs fall in this category. As discussed in this thesis metal-sulphide precipitates may be a major sink for some metals in anoxic wetlands. Noteworthy, these sulphide phases are amenable to undesirable premature dissolution during the SEP. Several studies demonstrated that hydroxylammonium hydrochloride may leach sulphide-bound metals so they can be construed as being bound to Fe-Mn (oxy)hydroxides (Lacal et al., 2003; Peltier et al., 2005; Burton et al., 2006). This reagent is used in the most commonly applied procedures, namely those based on the Tessier protocol (Tessier et al., 1979) and the original and modified BCR protocols (Quevauviller et al., 1994;

Rauret et al., 1999). Peltier et al. (2005) examined the speciation of Zn and Pb in anoxic wetland sediments using SEP based on a modified Tessier protocol and simultaneously performing X-ray absorption spectroscopy analysis to check the accuracy of the extraction results. The modifications of the original protocol (Tessier et al., 1979) included the use of *aqua regia* instead of HF-$HClO_4$ for the residual fraction, and the use of 0.04 M hydroxylammonium hydrochloride in the step targeting fraction bound to Fe-Mn oxides. The findings of Peltier et al. (2005) indicated that the sequential extraction method resulted in misidentification of approximately 50% of Zn bound to reducible Fe-Mn oxides, rather than sulphides. This early solubilisation of metals from sulphide phases was observed mostly during the step when hydroxylammonium hydrochloride is used. As mentioned above, this reagent is commonly used in many sequential extraction methods routinely used to examine metal speciation in sediments, thus its low selectivity may reduce applicability of these methods for anoxic sediments. It was suggested that the content of metals bound with sulphides would be underestimated particularly for samples with freshly formed metal sulphides (amorphous), such as those in wetland sediments. In samples where metal sulphides occur as primary minerals, such as sphalerite (ZnS) and galena (PbS), the results of sequential extraction seem to correspond well to metal speciation determined with other methods (Peltier et al., 2005).

Metal fractions in the optimised BCR protocol (BCR2)

The Community Bureau of Reference of the Commission of the European Communities (BCR) commissioned research which resulted in the development of a harmonized, three-stage, sediment sequential extraction protocol sometimes referred to as the BCR1 protocol (Quevauviller et al., 1994). In this protocol the two first extraction steps of the Tessier protocol (Tessier et al., 1979) were replaced by a single step. The other difference was that increased volumes of reagents and increased amounts of samples were recommended in the protocol, which allowed avoiding analytical problems associated with the use of small volumes of extractants, and also more representative sampling. The fractions targeted by the extractants were labelled according to the chemical process (affinity for the extractant) rather than specific mineral phases (Bacon and Davidson, 2008). The original protocol was reevaluated and published in a revised form (by Rauret et al., 1999) taking into account several limitations of the BCR1 protocol (Tab. 5.1). The revised protocol is often referred to as the BCR2 protocol (Bacon and Davidson, 2008).

Table 5.1 The BCR2 sequential extraction procedure (adapted from Rauret et al., 1999; Bacon and Davidson, 2008)

	Reagent	**Fraction label**	**Nominal target phase(s)**
Step 1	Acetic acid, 0.11 mol/L	Exchangeable, water and acid-soluble	Soluble and exchangeable cations and carbonates
Step 2	Hydroxylammonium hydrochloride 0.5 mol/L at pH 1.5	Reducible	Fe-Mn oxyhydroxides
Step 3	Hydrogen peroxide (85°C), 300 mg/g, 8.8 mol/L then ammonium acetate, 1.0 mol/L	Oxidisable	Organic matter and sulphides
Step 4	*Aqua regia*[1]	Residual	

[1]Although not officially a step in sequential extraction, it is recommended that the residue at the end of Step 3 be digested with *aqua regia* and the sum of the four fractions be compared with the results of a separate *aqua regia* digestion of the sample

The BCR2 allows distinction of three operationally-defined phases, which are determined in a step-wise manner using the extractants as specified in Tab. 5.1. The interpretation of each fraction is discussed below with particular focus on applications for substrates sampled from CWs. The fourth-step, in which *aqua regia* is applied, was added as a quality-assurance step and the targeted fraction is labelled as residual. This fraction was also discussed briefly below.

Exchangeable, water and acid-soluble fraction

The fraction that is extracted in step 1 of the BCR2 protocol covers three target phases: water soluble, exchangeable and acid-soluble. The exchangeable fraction corresponds to the forms of metals that can be easily available for plant uptake and can be released by merely changing the ionic strength of the contacting solution (Filgueiras et al., 2002). However, even elements that are extracted early in a sequential extraction are not necessarily currently bioavailable and may never become so (Bacon and Davidson, 2008). The extractant that is used in step 1 of the BCR2 protocol is 0.11 M acetic acid. The water-soluble fraction contains free ions and ions complexed with soluble organic matter and other constituents. Metals and metalloids within this fraction are the most mobile. The exchangeable fraction contains weakly adsorbed metals bound to the solid surface by relatively weak electrostatic forces, metals that can be released by ion-exchange processes and metals that can co-precipitate with carbonates. Remobilisation of metals belonging to this fraction may occur upon changes of the ionic composition (affecting adsorption-desorption equilibrium) or lowering of pH. Trace metals can be adsorbed on sediments or on their major constituents such as clay, hydrated Fe-Mn oxides, and humic acids. Several studies have shown that significant proportion of trace metals can be associated with carbonates in the sediments. This is a case especially when Fe-Mn oxides and organic matter are less abundant.

The carbonate fraction is weakly bound phase and prone to change with environmental conditions. The metal content bound to carbonates is particularly sensitive to pH changes and can become mobile when pH is sufficiently low (Filgueiras et al., 2002; Song, 2003). Noteworthy, on contact with air anoxic sediments become acidified, which may lead to dissolution of carbonate content and subsequent release of metals such as Zn, Cd, Ni, Mn and Ca (Buykx et al., 2000). The reagent used in this step is capable of dissolving carbonates and dolomite without significant extraction of metals bound to organic matter, Fe-Mn oxides and aluminosilicates. Significant amount of Fe and Mn extracted from sediments can be attributed to solubilization of $FeCO_3$ and $MnCO_3$ in acidic solutions of the reagent rather than to the attack of Fe-Mn oxides. Under more acidic conditions, however, the solubilization of Fe-Mn oxides can take place, and the associated metals (e.g. Cu, Pb, Zn, etc.) are released. Under soil conditions, metals within the exchangeable and acid-soluble fractions are considered readily and potentially bioavailable, while the reducible and oxidisable fractions are relatively stable (Filgueiras et al., 2002).

Reducible fraction

Hydrous Fe-Mn oxides exist in large proportion in soil and sediments as nodules, concretions, cement between particles, or as coating on particles. Scavenging of trace metals by these oxides can occur by any or a combination of the following mechanisms: co-precipitation, adsorption, surface complex formation, ion exchange, and penetration of the lattice. They have high affinity for trace metals and are thermodynamically unstable under anoxic conditions and are attacked by benthic organisms. Reduction of oxidized species of Fe^{III} and Mn^{IV} under anoxic circumstances and resultant dissolution may lead to a release of associated trace metals. The BCR2 protocol does not differentiate between sub-fractions into which the reducible fraction can be divided: easily reducible fraction (Mn oxides), moderately reducible fraction (Fe oxides) and poorly reducible fraction (crystalline Fe oxides) (Filgueiras et al., 2002; Song, 2003). Some protocols allow distinction between these sub-fractions e.g. Miller et al. (1986), Krishnamurti et al. (1995). In the BCR2 protocol the reagent that is employed for extraction of the reducible fraction is hydroxylammonium hydrochloride at pH 1.5. It was reported that this reagent overestimates the amount of metals in the oxide fraction at the expense of the oxidisable fraction (mostly organic matter). The pH value seems to be crucial as below 1.5 the reagent may partly leach the metal content associated with silicate fraction (Tessier et al., 1979).

Oxidisable fraction

Trace metals may be bound to various forms of organic matter such as living organisms, detritus, coatings on mineral particles, or organic bed media (peat or compost) employed for various purposes. The binding forces include complexation and bioaccumulation processes. Under oxidizing circumstances degradation of organic matter may result in a release of soluble trace metals bound to it (Filgueiras et al., 2002; Song, 2003). Trace metals precipitated as sulphides may also be leached during this extraction step (Marin et al., 1997). The organic fraction released in the oxidisable step is an important fraction as it can dominate trace metal distribution in CWs. The oxidation process that is used in the BCR2 protocol is mediated by hydrogen peroxide in acid medium heated to 85°C. After the oxidation step ammonium acetate is added to avoid metal readsorption in the residual fraction and to extract metals. According to Sutherland et al. (2000) oxidation of resistant organics and sulphide materials may not be complete in the presence of high organic matter content.

Residual fraction

The metal fraction associated with the residual fraction (e.g. silicate) can only be mobilised as a result of weathering (Filgueiras et al., 2002). However, when *aqua regia* is used, which is recommended in the BCR protocol, the contribution of silicate-bound metals is negligible, and may be rather attributed to primary and secondary minerals and trace metals bound within their crystal structure (Song, 2003; Peltier et al., 2005).

5.1.3 Metal removal mechanisms

The important feature of metals is that they can only be removed from wastewater but cannot be destroyed. This allows assumption that metals are accumulated in wetland substrate or biota (Ranieri and Young, 2012).

The removal of metals in subsurface CWs is dominated by four mechanisms:

- adsorption;
- filtration and sedimentation;
- association with metal oxides and hydroxides;
- precipitation as sulphides.

The extent of these processes depends on the type of the CWs, the pH, redox potential, influent composition, the dominant plant species and microbial activity (Kosolapov et al., 2004).

Adsorption

In the wetland substrates metals can be adsorbed by cation exchange (physical adsorption) or chemisorption. Chemisorption is a stronger and more permanent form of binding than cation exchange. The metal removal mechanism is dependent on the adsorption process especially in the initial period of a CW's operational lifetime before sorption sites are not saturated (Sheoran and Sheoran, 2006). The adsorption of metals onto organic matter is mediated by the carboxyl and phenolic hydroxyl residues of molecules (e.g. humic acids) produced or exposed during the decomposition of plants. Further degradation of this detrital matter may lead to more stable chelate complexes. Copper and Ni have been shown to have particular affinity to organic matter and to be retained by adsorption in wetlands (Sobolewski, 1999). It was observed that metals may bind to functional groups of organic matter adsorbed on Fe hydr(oxides), in particular in acidic conditions (Song, 2003). Adsorption depends on pH as functional groups responsible for metal sorption such as carboxylic and phenolic groups are deprotonated at high pH and become available for binding dissolved metals (Sheoran et al., 2010).

Filtration and sedimentation

Settling and sedimentation are responsible for efficient removal of heavy metals associated with particulate matter (Sheoran and Sheoran, 2006). The filtration and sedimentation are responsible for the removal of precipitates of the external origin and those formed in the CWs. For example, residual metal hydroxides present in the electroplating wastewater may be entrapped in the bed media of CWs. Also the efficiency of metal removal as sulphides or (oxy)hydroxides depends to some extent on the filtration and sedimentation processes (Sheoran et al., 2010).

Association with metal oxides and hydroxides

Aluminium, Fe and Mn may form insoluble compounds in the CWs by oxidation (abiotic or bacterially-mediated) or hydrolysis according to the simplified reactions (Sobolewski, 1999):

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+ \quad (5.1)$$

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (5.2)$$

$$Fe^{3+} + 2H_2O \rightarrow FeOOH + 3H^+ \quad (5.3)$$

$$Mn^{2+} + 0.25O_2 + 1.5H_2O \rightarrow MnOOH + 2H^+ \quad (5.4)$$

The most common reaction for Al removal is precipitation as hydroxide (PIRAMID, 2003). If dissolved sulphate is present Al may also precipitate as hydroxysulphate (Kadlec and Wallace,

2009). Solubility of Al is dependent only on the pH of water and is unaffected by the oxidation and reduction processes that govern the mobility of other metals (Sobolewski, 1999).

In an oxygenated environment ferric iron is present as insoluble oxydroxides, denoted as FeOOH (Kadlec and Wallace, 2009). In fact, a variety of Fe oxides, hydroxides and oxyhroxides are formed in CWs. The presence of Fe^{II} causes reduction of oxidized manganese precipitates (e.g. MnO_2 and MnOOH) (Sobolewski, 1999). Oxidation of Mn is much slower than for iron and can be markedly accelerated by bacteria (Sheoran and Sheoran, 2006). Iron and Mn oxides and (oxy)hydroxides are considered excellent scavengers for other metals such as Cu, Ni, Pb and Zn (Kosolapov et al., 2004; Kadlec and Wallace, 2009). Trace metals may become associated with oxidized forms of Fe or Mn (oxy)hydrooxides due to adsorption or co-precipitation phenomena, which reflect both general (cation-exchange) and specific (iron-arsenic co-precipitation) reactions (Sobolewski, 1999). For example, 97% of Zn was found to be bound to hydroxide phase in the top layer of sediments (Bostick et al., 2001; Kadlec and Wallace, 2009).

Manganese sulphide is stable only under very basic conditions. The major removal mechanisms of Mn are precipitation and co-precipitation as or with oxides and (oxy)hydroxides. Iron and Mn may be introduced into the CW in the influent or may be leached from the substrate under anoxic conditions. These metals precipitate as oxides, oxyhydroxides, or hydroxides in the oxidizing (micro)environment of the wetland system. When wetland substrate becomes reducing, the metals bound to Fe and Mn oxides and (oxy)hydroxides can be remobilized causing their massive release. The presence of oxidized Fe can inhibit bacterial sulphate reduction when amorphous Fe^{III} is present. Bacteria capable of reducing ferric iron can outcompete sulphate-reducing and methanogenic bacteria for electron donors. The inhibition of sulphate reduction stems also from the fact that the redox conditions in the substrate are buffered above the range required for this process (Reddy and DeLaune, 2008). Highly efficient sinks of trace metals can be rhizoconcretions on the roots commonly known as iron plaque. The structures may contain 5–10-fold more trace metals then the adjacent substrate.

Precipitation with sulphides

Sulphate-reducing bacteria (SRB) mediate dissolved sulphate reduction to hydrogen sulphide and metals are immobilized as sulphide minerals according to the reactions:

$$SO_4^{2-} + 2CH_2O(org) \Rightarrow H_2S + 2HCO_3^- \quad (5.5)$$

$$H_2S + Me^{2+} = MeS(s) + 2H^+ \quad (5.6)$$

where Me is divalent metal cation. It can be seen that reaction (5.5) produces 2 moles of bicarbonate, the acidity produced by reaction (5.6) tends to offset this, thereby protons are conserved in the overall process (Gammons and Frandsen, 2001):

$$SO_4^{2-} + Me^{2+} + 2CH_2O(org) = MeS(s) + 2H_2CO_3(aq) \quad (5.7)$$

The thermodynamic equilibria of metal sulphide precipitation are as follows (Lewis, 2010):

$$H_2S \Leftrightarrow HS^- + H^+ \quad K_{p1} = \frac{[HS^-][H^+]}{[H_2S]}, \quad pK_1 = 6.99 \quad (5.8)$$

$$HS^- \Leftrightarrow S^{2-} + H^+ \quad K_{p2} = \frac{[S^{2-}][H^+]}{[HS^-]}, \quad pK_2 = 17.44 \quad (5.9)$$

$$Me^{2+} + S^{2-} \Leftrightarrow MS(s) \quad (5.10)$$

$$Me^{2+} + HS^- \Leftrightarrow MeS(s) + H^+ \quad (5.11)$$

$$2Me^{+} + HS^- \Leftrightarrow Me_2S(s) + H^+ \quad (5.12)$$

These equations are simplified and may not be true for non-stoichiometric forms and diagenetic MeS_2 forms, such as the Fe sulphides greigite and pyrite. It is noteworthy that sulphide precipitation is a multistage process, which initially consists in deposition of non-stoichiometric phases that subsequently undergo diagenetic reorganization to crystalline forms. Sulphides are (meta)stable under anaerobic conditions but undergo decomposition at the oxic-anoxic interface, which leads to release of covalently bound metals and co-precipitated and adsorbed metals or metalloids (Lens et al., 2007). In general metal sulphides are less soluble than their carbonate or hydroxide counterparts, achieving more complete precipitation and stability over a broader pH range (Tab. 5.2) (Blais et al., 2008). It can be stated that the solubility of metal sulphides is extremely low providing that anaerobic conditions prevail. In a CW or a reactor with suspended SRB biomass it can be observed that the production of H_2S exceeds its consumption by metal precipitation. Excessive amounts of H_2S (or HS^-) may adversely affect the environment (toxicity to aquatic live and foul smell of gaseous form) and increase metal mobility in the system. The latter stems from the fact that HS^- may form stable aqueous compounds with metals. For example, if HS^- concentration is above 0.1 mol/L the dominant copper species is $CuS(HS)_3^{3-}$, and the $CuS(HS)_2^{2-}$ does not become significant until the HS^- concentration limit fall below 0.2 mol/L.

In general the redissolution of precipitated metal sulphides by excessive sulphide is described by the reaction (Lewis, 2010):

$$MeS(s) + HS^{-}(aq) \Leftrightarrow MeS(HS)^{-}(aq) \quad (5.13)$$

Removal of metals in sulphate-reducing wetlands was reported to follow two mechanisms: the adsorption of metal onto organic matter and the formation of metal-sulphide precipitates. Adsorption onto organic matter appears to occur much more rapidly than sulphide precipitation. Metals absorbed to organic matter appear to convert slowly to sulphide precipitates (Wallace and Knight, 2006; Fitch and Burken, 2003). Also the SRB biofilm is able to attract metals and bind them by several mechanisms as it is mentioned below. The key requirements for sulphate-reducing systems are: anaerobic conditions (oxidation-reduction potential below -100 mV), electron donors (simple organic compounds), microbial groups capable of utilizing inorganic sulphur compounds as electron acceptors; inorganic sulphur compounds (as electron acceptors) (Younger et al., 2002; PIRAMID, 2003; Reddy and DeLaune, 2008). In freshwater wetlands, sulphate reduction rates are generally limited by the amount of sulphates. In CWs, however, sulphate concentration is usually greater because of wastewater loading, and the sulphate reduction rates are mostly dependent on the substrate supply. Therefore, a major factor limiting the application of microbial sulphate reduction to the removal of metals from carbon deficient industrial wastewaters in wetland systems is the availability of carbon and energy sources to drive the process. In CWs, for sulphate reduction to be effective for treating wastewater, factors which promote the process and sulphide formation must be maximized and destructive factors minimized. First of all, in order to stimulate the sulphate-reducing microorganisms in the case of carbon-deficient effluents, a proper carbon source should be provided to enhance their growth and to cause other bacteria to remove the oxygen from the environment (Kosolapov et al., 2004). This aspect is discussed in Section 5.4.3. Some of the metals which react with hydrogen sulphide to form highly insoluble metal sulphides are: Cu, Fe, Ni, Pb, and Zn. These metals are often present in electroplating wastewater (Wallace and Knight, 2006). Precipitation of metals with sulphides has several advantages when compared with precipitation by hydroxides: (i) the residual metal concentrations in the effluent are lower; (ii) the interference of the chelation agents in wastewater is less problematic; (iii) precipitation offers increased selectivity; (iv) higher reaction rates result from lower hydraulic retention time (Blais et al., 2008).

There are several processes that are related to bacterial sulphate reduction. It was found that SRB have broad-specificity enzymes that are able to reduce the oxidation state of metals or metalloids, thereby reducing their solubility. The SRB cells and associated extracellular

polymers are responsible for: biosorption and bioconcentration of soluble ions, nucleation of precipitate at biopolymer surfaces, and electrostatic entrapment of particulates. These processes may contribute directly to the removal of metals but may also enhance efficiency of the related processes as sulphide precipitation (Lens et al., 2007). The chemical reduction of metals can be attributed to enzymatic action of bacteria but may also occur as abiotic process when the conditions in the system are sufficiently reducing. For example, *Desulphovibrio* group of SRB may reduce Fe^{III} to Fe^{II}, and Cu^{II} is reduced to Cu^{I} in sulphidic solutions (Lens et al., 2007; Lewis, 2010). Interestingly, Cu may also be reduced to its metallic form under slightly acidic and reduced conditions when the amount of sulphide is low (Sobolewski, 1999).

Not all metals may precipitate as sulphides. The examples are: Al and Mn, which may occur in electroplating wastewater. In the reducing conditions associated with sulphate reduction it is suspected that Al may precipitate (as alunite) according to the reaction:

$$3Al^{3+} + K^{+} + 6H_2O + 2SO_4^{2-} \rightarrow KAl_3(OH)_6(SO_4)_2 + 6H^{+} \quad (5.14)$$

Role of plants in the removal of metals

The major sink of metals in CWs can be their vegetation or substrate, depending on the hydraulic properties of the system (Lesage, 2006). Only a minor fraction of metals is removed by emergent hydrophytes in the subsurface-flow CWs (Marchand et al., 2010). The wetland plants that are able to accumulate metals enabling their efficient removal are especially floating (e.g. *Eichhornia crassipes*, *Lemna minor*) or submersed species (e.g. *Myriophyllum spicatum*). The use of water hyacinth (*Eichhornia crassipes)* is in general limited to warm weather regions because of its sensitivity to freezing conditions (Kadlec and Wallace, 2009). Although the systems with duckweed (*Lemna minor*) were more common in colder climates (e.g. 35 systems in Poland as of 2003), their poor and erratic performance precludes consideration for further implementation (Vymazal and Kröpfelová, 2008). In general, there is a concern regarding ecotoxicological threat and disposal of these plants. High levels of the accumulated metals pose a risk of their accumulation in the food chain by direct consumption or decomposition. Also the use of the harvested biomass remains unresolved (Lesage, 2006; Vymazal and Kröpfelová, 2008). The application of submersed plants for the accumulation of metals was not well studied (Lesage, 2006). In the CWs of subsurface flow the role of the plans in metal removal consists in:

- enhancing hydraulic conductivity of the bed media,
- providing additional surface area for microorganisms, adsorption and surface-catalysed, oxidative precipitation of metals,

- providing carbon source for bacteria.

The vegetation may change redox potential of the wetland substrate and thereby affect metal mobility. The diffusion of oxygen from the roots may exert both negative and positive effect on the stability of metals in the substrate. The oxygen released by the roots may cause dissolution of metal sulphides previously precipitated in the CW and increase acidity of the water in the system posing a serious environmental threat. On the other hand, when the amount of sulphides is negligible, the oxygen release by the roots may induce microbial oxidation and precipitation of iron (oxy)hydroxides, which may bind other metals. The remobilization of metals in the substrate may also result from acidification by organic exudates excreted by plants. These substances along with other organic plant-related material (due to senescence or root tissue sloughing) may stimulate the growth of heterotrophic bacteria (Ye et al., 2001b; Kosolapov et al., 2004).

Other processes for removal of metals

The other processes that may play a role in the removal of metals can be: formation of carbonates, metals hydrolysis (catalysed by bacteria under acidic conditions), reduction to non-mobile forms (biologically or chemically), biological methylation (and subsequent volatilization; especially for mercury) (Sobolewski, 1999; Obarska-Pempkowiak et al., 2010). It is also possible that metals could be complexed by organic ligands, such as acetate. This phenomena, however, cannot occur under typical conditions prevailing in wetlands (Gammons and Frandsen, 2001). Aluminium may also precipitate with phosphate (Kadlec and Wallace, 2009). Probably, the most complex chemistry in natural system is observed for Pb, which apart from sulphides, carbonates and hydroxides may form oxide (PbO), sulphate ($PbSO_4$), charged hydroxide ($PbOH^+$), and biplumbite ion ($HPbO_2^-$) (Reddy and DeLaune, 2008; Lewis, 2010). The summary of the processes responsible for the removal of metals in the subsurface flow CWs are listed in Tab. 5.2.

Table 5.2 Overview of the dominant processes responsible for metal removal in subsurface flow CWs

	Al	Cu	Fe	Ni	Pb	Zn
Adsorption onto organic matter		+	+	+	+	+
Co-precipitation as/with (oxy)hydroxides	+	+	+	+	+	+
Precipitation as sulphides		+	+	+	+	+
Precipitation as carbonates		+	+	+	+	+
Precipitation with phosphates	+		+			

The chemistry of metals in wetlands is complex and numerous mechanism may play a role in the removal of metals. The interplay between various biotic and abiotic processes has not been well

understood and needs further investigations (Gammons and Frandsen, 2001). Based on the solubility product constant (K_s) of different metal species (Tab. 5.3) it is sometimes assumed that the removal of metals as sulphides is the most advantageous process, as in general sulphides are characterized by the lowest solubility as compared to hydroxides and carbonates. However, the mobility of metals in sulphidic systems is often underestimated as the solubility of freshly precipitated amorphous forms of metal sulphides can be several times higher than for the crystalline form (which are shown in Tab. 5.3) (Gammons and Frandsen, 2001). Thus, straightforward application of the solubility data cannot explain trace metals levels in natural systems (Reddy and DeLaune, 2008).

Table 5.3 Solubility products of selected metal sulphides, hydroxides and carbonates (adapted from Blais et al., 2008)

Element	Sulphide		Hydroxide		Carbonate	
	Form	**log K_s**[1]	**Form**	**Log K_s**	**Form**	**log K_s**
Al			$Al(OH)_3$ alpha	-33.5		
Cu	Cu_2S	-47.6	Cu(OH)	-14.7	$CuCO_3$	-11.5
	CuS	-35.2	$Cu(OH)_2$	-18.6		
Fe	FeS	-17.2	$Fe(OH)_2$	-15.1	$FeCO_3$	-10.2
	Fe_2S_3	-85.0	$Fe(OH)_3$	-37.4		
Mn	MnS pink	-9.6	$Mn(OH)_2$	-12.7	$MnCO_3$	-10.6
	MnS green	12.6				
Ni	NiS alpha	-18.5	$Ni(OH)_2$	-14.7	$NiCO_3$	-6.9
	NiS gamma	-25.7				
Pb	PbS	-27.0	$Pb(OH)_2$	-16.1	$PbCO_3$	-13.1
Zn	ZnS sphalerite	-23.0	$Zn(OH)_2$	-15.7	$ZnCO_3$	-10.0
	ZnS wurtzite	-24.3			$ZnCO_3 \cdot H_2O$	-10.3

[1] Ks solubility product constant

5.2 Boron

5.2.1 Chemistry of boron

Boron is a metalloid that occurs naturally as borosilicates, boric acid, borax, and other borate minerals in the oceans, rocks, coal, shale and in some soils, from which is released into the atmosphere and aquatic environment. The mobilized borosilicate structures can form stable species like boric acid and polyanions, which are biologically active. The anthropogenic sources of boron are detergents and bleachers and also those related to glass production, mining and agriculture (Davis et al., 2002). Boron may be also present in electroplating wastewater as it is frequently used in autocatalytic electroless nickel-boron plating on light alloys (with an alkaline bath containing sodium borohydride as the reducing agent) (Delaunois et al., 2000). Boron is a

micronutrient that is essential for normal growth and development of plants. It is also essential for small aquatic organisms, such as diatoms (Powel et al., 1997; Kadlec and Wallace, 2009).

5.2.2 Fate of boron in constructed wetlands

Main removal mechanism for B is adsorption on: clay particles, crystalline and amorphous iron and aluminium oxides, and peat. Removal of boron is higher for elevated concentrations in industrial, mining and leachate waters, and lower or negative for stormwaters and municipal wastewaters (Kadlec and Wallace, 2009). The removal efficiency for leachate from coal combustion by-products was in the range of 12–28% (Hoover et al., 1998; Ye et al., 2001ab) and for construction debris leachate treated in vertical peat filter it was 91% (Sartaj et al, 1999), which is believed to be the maximum value reported in the literature (Kadlec and Wallace, 2009).

5.3 Cyanides

5.3.1 Chemistry of cyanides

Cyanide compounds contain the cyano-moiety consisting of the carbon atom linked to the nitrogen atom (−C≡N) by triple bond. Hydrogen cyanide (H−C≡N), hydrocyanic acid is the most basic, and the most toxic, of these compounds. HCN is a gas at ambient temperature, and is freely soluble in water. In water, HCN dissociates at high pH (pK_a = 9.24 at 25°C) to form the cyanide anion, CN^- (Wong-Chong et al., 2006). Cyanide may occur in many different forms in water and soil systems. The cyanide species determines its environmental fate and transport of cyanide, as well as its toxicity (Ghosh et al., 2006a). In water cyanide may occur as free cyanide, metal cyanide complexes, cyanate, thiocyanate, and organocyanides. The cyanide anion may react with many metal cations to form metal–cyanide complexes. These species are typically anionic and have a general formula of $Me(CN)_x^{n-}$, where Me is a metal cation, *x* is the number of cyanide groups, and *n* is the ionic charge of the metal-cyanide complex. Metal-cyanide complexes vary in their strength of bonding and based on that property can be divided into two groups: weak metal-cyanide complexes and strong metal-cyanide complexes. In weak metal-cyanide complexes the cyanide ions are weakly bonded to the metal cation and because of that they dissociate under mildly acidic conditions (pH = 4 to 6) to produce free cyanide. On the other hand, strong metal-cyanide complexes include cyanide complexes with transition metals (e.g. iron, platinum, cobalt, gold) and require strong acidic conditions to dissociate and form free cyanide. Owing to that, those complexes are more stable in aqueous solutions and relatively less toxic (Ghosh et al., 2006a). Summary of the above-mentioned cyanide species and other

compounds that may occur in electroplating wastewater and during the treatment in CWs is given in Tab. 5.4.

Table 5.4 Commonly occurring aqueous cyanide species (adapted from Ghosh et al., 2006a)

Classification	Cyanide species
Free cyanide	HCN, CN^-
Weak metal-cyanide complexes	$Cu(CN)_2^-$, $Cu(CN)_3^{2-}$, $Cu(CN)_4^{3-}$,
	$Ni(CN)_2^0$, $Ni(CN)_3^-$, $Ni(CN)_4^{2-}$, $Ni(CN)_4^-$, $Ni(CN)_4^0$, $Ni(CN)_4^+$
	$Zn(CN)_2^0$, $Zn(CN)_3^-$, $Zn(CN)_4^{2-}$
Strong metal-cyanide complexes	$CaFe(CN)_6^{2-}$, $CaFe(CN)_6^-$, $Ca_2Fe(CN)_6^0$, $CaHFe(CN)_6^{2-}$,
	$Fe(CN)_6^{4-}$, $HFe(CN)_6^{3-}$, $H_2Fe(CN)_6^{2-}$, $Fe_2(CN)_6^0$,
	$K_2H_2Fe(CN)_6^0$, $K_3HFe(CN)_6^0$, $K_3HFe(CN)_6^{2-}$,
	$K_2Fe(CN)_6^{2-}$, $KFe(CN)_6^{3-}$,
	$Fe(CN)_6^{3-}$,
	$MgFe(CN)_6^-$, $MgFe(CN)_6^{2-}$,
	$NH_4Fe(CN)_6^{3-}$, $(NH_4)_2Fe(CN)_6^{2-}$, $NH_5Fe(CN)_6^{2-}$,
	$NaFe(CN)_6^{3-}$, $Na_2Fe(CN)_6^{2-}$, $NaHFe(CN)_6^{2-}$,
	$Au(CN)_2^-$,
	$Pt(CN)_4^{2-}$
Cyanate	HOCN, OCN^-
Metal-cyanate complexes	$Ag(OCN)_2^-$, and others
Thiocyanate	HSCN, SCN^-
Metal-thiocyanate complexes	$MgSCN^+$,
	$MnSCN^+$,
	$FeSCN^+$,
	$FeSCN^{2+}$, $Fe(SCN)_2^+$, $Fe(SCN)_3^0$, $Fe(SCN)_4^-$, $FeOHSCN^+$,
	$CuSCN^+$, $Cu(SCN)_2^0$,
	$NiSCN^+$, $Ni(SCN)_2^0$,
	$ZnSCN^+$, $Zn(SCN)_2^0$, $Zn(SCN)_3^-$, $Zn(SCN)_4^{2-}$, and others
Organocyanides	Nitriles (e.g., acetonitrile) Cyanohydrins Cyanocobalamin and others

5.3.2 Removal mechanisms of cyanides

Various physical-chemical techniques are applied in electroplating wastewater treatment for the removal of cyanides. Alkaline chlorination method is commonly used for the treatment of cyanide compounds in industrial wastewater. The shortcomings of this method are that it is not effective in the case of strong metal-cyanide complexes (Dash et al., 2009). For instance, Fe-cyanide complexes are very stable and not materially toxic, however, they are amenable to rapid

and extensive photolysis when exposed to UV radiation (Eaton et al., 2005). Thus, even treated electroplating wastewater may pose environmental threat. CWs cannot compete with the conventional methods for the removal of cyanide compounds, but they might be a promising solution to removal of residual cyanide species. The processing of cyanide in wetlands is known to involve microbial degradation, volatilization, plant uptake, and complexation with iron (Kadlec and Wallace, 2009). Cyanides can be destroyed by both aerobic and anaerobic microorganisms. Additionally, some plants are able to digest cyanides and degrade it (Gessner et al., 2005). The advantage of using CWs for polishing or treatment of wastewater containing cyanide is that they offer various concomitant processes leading to destabilization or degradation of cyanide species (Fig. 5.1).

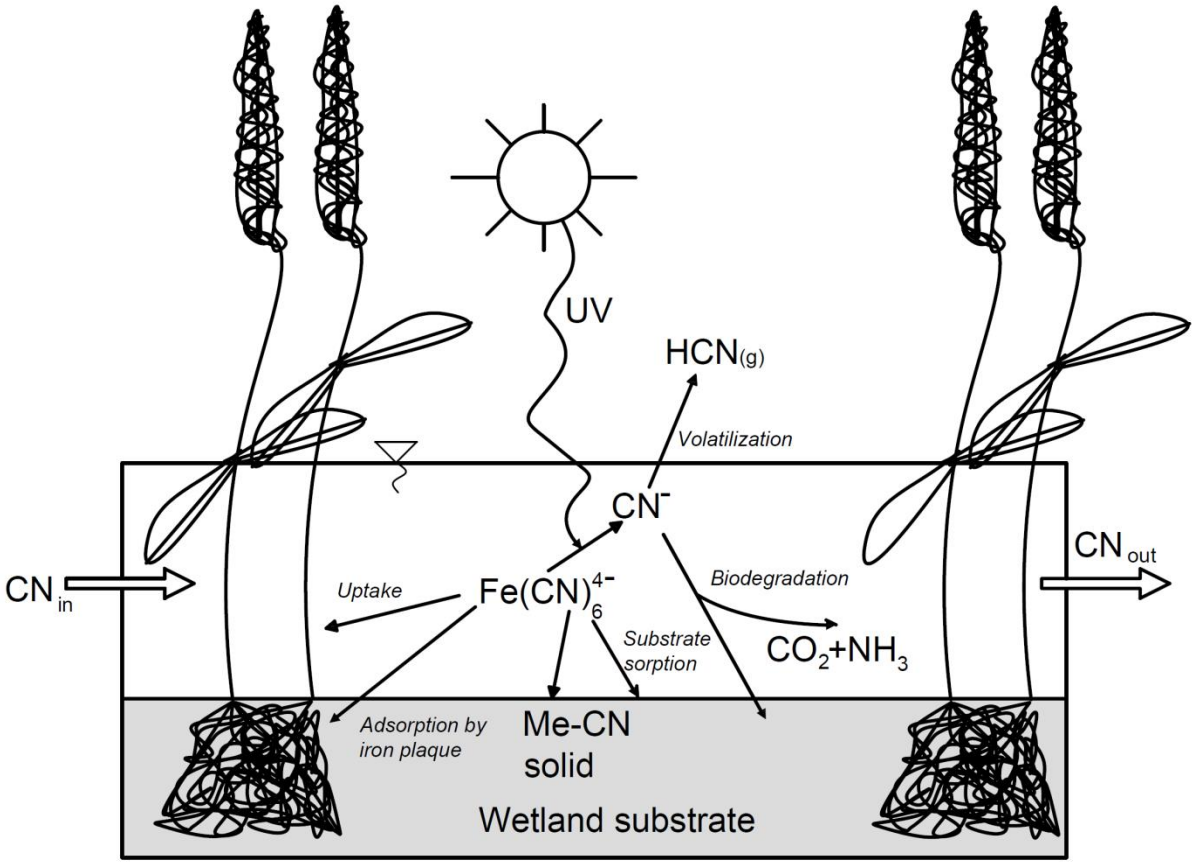


Figure 5.1 Cyanide attenuation mechanism in constructed wetland (adapted from Bushey, 2003; Ghosh et al., 2006b)

Constructed wetlands because of their features offer a wide array of biotic and abiotic processes that may play a role in degradation of cyanides: (i) transport processes (advection and dispersion); (ii) photolysis (dissociation of iron/other metal-cyanide complexes to free cyanide via UV irradiation); (iii) volatilization of free cyanide into the atmosphere; (iv) microbiological

degradation of free cyanide and (to limited degree) complexed cyanide; (v) plant uptake and assimilation of free and metal-complexed cyanide; (vi) rhizosphere mediated degradation of cyanide species; (vii) adsorption of free and metal-cyanide complexes onto sediments; (viii) precipitation/dissolution of iron and other metal-cyanide complexes (Ghosh et al., 2006b). Cyanide complexes with Fe or Au are less biodegradable than free cyanides and weak metal-cyanide complexes. As it can be seen in Fig. 5.1 cyanide complexes with Fe undergo photolysis in the presence of UV light to yield free cyanide. In the dark, however, they dissociate slowly. For this reason, many studies failed to prove conclusively that removal of Fe-cyanide complexes was attributed to bacteria or plants and not to the physical-chemical phenomena. It is believed that bacteria *Pseudomonas fluorescens* and *Fusarium solani* can degrade these complexes. Additionally, a plant-mediated metabolism of ferrocyanide was observed in the study with willow (*Salix eriocephala*) (Ebbs, 2004; Dash et al., 2009). The bacterial pathway for the degradation of Fe-cyanide complexes is not indicated in Fig. 5.1. as no evidence exists for occurrence of this mechanism in wetlands.

Gessner et al. (2005) investigated the use of pilot SF CWs for the reduction of complex and free cyanide and associated pollutants in water from a groundwater spring in the area of historical aluminium industry landfills. Shallow basins, initially planted with cattail (*Typha latifolia*) and bulrush (*Schoenoplectus tabernaemontani*), subsequently converted to coontail (*Ceratophyllum demersum*) and pondweed (*Potamogeton spp*.). Both total and free cyanide were effectively reduced during 7-d retention, by 56% and 88%, respectively. Alvarez et al. (2006a; 2006b) carried out experiments with wetland passive systems at laboratory and field scale at the site of a gold mine in northern Spain. The pilot-scale system vegetated with *Typha latifolia* (unplanted control systems were also tested) included aeration cascades and aerobic and anaerobic cells. The results of these studies suggest that this technology was able to treat wastewaters with about 25% reduction of dissolved cyanide at nominal retention of 6 d. The waste strengths in this study were relatively high, with total cyanide of about 200 mg/L and dissolved cyanide of 75 mg/L. The removal of weak metal-cyanide complexes was 99%, from 15 mg/L to 0.14 mg/L. Garcia (2003) found more than 90% reduction in wetland mesocosms planted with *Typha*. Halford (1999) in a large-scale CW system covered with peat and planted with *Typha orientalis* observed the removal of total cyanide with the efficiency between 80–98% and the removal of weak metal-cyanide complexes about 78%.

5.4 Occurrence of sulphur in constructed wetlands

Although, sulphates are released in large quantities to the environment the environmental risk caused by their presence is relatively low compared to other contaminants. Sulphate pollution may cause, however, several indirect environmental effects. Excessive quantities of sulphate may exert adverse effect on public water supplies and pose threat to life forms (Liamleam and Annachhatre, 2007). The source of sulphates in metal plating is mainly sulphuric acid used for adjusting pH of wastewater and as a component of bath solutions. It was reported in the literature that concentrations of sulphates in electroplating wastewater in the range of 100–1000 mg/L are common (Pümpel et al., 1999; Bartkiewicz, 2006). In reducing conditions, sulphate acts as an electron acceptor and is reduced to sulphide. This phenomenon may be regarded as a threat but also as a means of reducing metal and sulphate pollution. The former aspect is discussed in more detail in Section 5.1.3. The adverse effect of reduced sulphur species is their odiferous nuisance and corrosive effect. Moreover, H_2S is toxic to humans. Health effects that have been observed in humans following exposure to hydrogen sulphide include death (exposure > 700 mg/m^3) and respiratory, ocular, neurological, cardiovascular, metabolic, and reproductive effects. The odour threshold for H_2S is 0.011 mg/m^3 (Chou, 2003). The production of H_2S and other gaseous sulphur compounds in wetlands may decrease their aesthetic values. Two other types of S gases emitted by wetlands, either constructed or natural, are sulphur dioxide (SO_2) and volatile organic sulphur compounds. All of these gases differ in their toxicity, the way they are produced, odour threshold and odour characteristics. SO_2 can be produced on the interface between anoxic and oxic compartments of a wetland where sulphides are oxidized on contact with air or by sulphide oxidizing bacteria. Some of the volatile organic sulphur compounds are: dimethyl sulphide, dimethyl disulphide, methyl mercaptane (methanethiol), carbonyl sulphide, and carbon disulphide. It is believed that each wetland has its own particular odour footprint depending on the type and quantity of the emitted gases (Hicks and Lemontagne, 2006). The amount of reduced sulphur species produced in reducing zones of a CW can be reoxidized chemically or biochemically. Bacterial oxidation of reduced sulphur species may occur under oxic conditions (by chemolithotrophic organisms such as *Acidithiobacillus*) or under anoxic conditions (by filamentous organisms such as *Beggiatoa*). Sulphide can also be oxidized by phototrophic process mediated by photosynthetic organisms such as *Chromatium* and *Chlorobium* (Faulwetter et al., 2009).

5.4.1 Bacterial sulphate reduction

Removal of metals is one of the useful applications of biological sulphate reduction. Sulphide generated by bacteria is used to chemically precipitate metals as sulphides. Insoluble metal sulphides allow removal of various metals from wastewater (Liamleam and Annachhtare, 2007). Sulphate reduction may occur through either assimilatory or dissimilatory pathways. In the assimilatory pathway oxidized forms of sulphur are reduced to generate sulphide, which is eventually transferred to amino acids as sulphhydryl groups (R-SH). Most of the reduced sulphur is fixed within the cell and only a small portion is released as volatile-reduced sulphur gases. This process occurs in both aerobic and anaerobic conditions. Nonetheless, upon death and decay organisms release the sulphur in the reduced state, which can result in the accumulation of high levels of hydrogen sulphide in wetland sediments or which can be reoxidized to elemental sulphur and sulphate in anaerobic and aerobic environments, respectively (Kadlec and Wallace, 2009; Reddy and DeLaune, 2008; Tang et al., 2009).

Dissimilatory sulphate reduction is mediated by SRB, this is, prokaryotic microorganisms, both bacteria and archaea, which can use sulphate as the terminal electron acceptor in their energy metabolism (Thauer et al., 2007). Majority of the SRB fall into one of the four phylogenetic lineages: (i) the mesophilic δ-proteobacteria with the genera *Desulfovibrio, Desulfobacterium, Desulfobacter,* and *Desulfobulbus*; (ii) the thermophilic Gram-negative bacteria with the genus *Thermodesulfovibrio*; (iii) the Gram-positive bacteria with the genus *Desulfotomaculum*; and (iv) the *Euryarchaeota* with the genus *Archaeoglobus* (Castro et al., 2000). Existence of a fifth, novel lineage of SRB, the *Thermodesulfobiaceae,* was reported by Mori et al. (2003). Many SRB are versatile in the ability to use different electron acceptors than sulphate. These include: elemental sulphur, fumarate, nitrate, dimethysulphoxide, Fe^{III}, Mn^{IV}. The group of microorganisms referred to as SRB include heterotrophic and autotrophic bacteria. Heterotrophic sulphate reducers use organic substances as substrates and autotrophs use CO_2 as a carbon source and H_2 as an electron donor (Thauer et al., 2007). The composition of SRB community may have an effect on the efficiency of metal sulphides precipitation. Thus the choice of inoculum may prove to be a key design aspect, however, it was not studied in detail (Webb et al., 1998).

5.4.2 Interactions between sulphate-reducing bacteria and other groups of bacteria

Methanogens and SRB share many ecological and physiological similarities and they often coexist in reducing zones of CWs. When the source of electron donor is not limited both methanogens and SRB can thrive because competition is greatly reduced (Khanal, 2008). Under

substrate-limiting conditions, both groups of bacteria compete for acetate and H_2. There are several factors that govern this competition, e.g. pH, COD/SO_4^{2-} ratio, substrate type or temperature. Methanogens and SRB may also be synergistically related when both groups rely on each other for their metabolic activities. Methanogens may utilize acetate or H_2 produced by SRB, and SRB may oxidize methane produced by methanogens (Khanal, 2008).

SRB are dependent on the other groups of bacteria when only complex organic matter is available (e.g. peat or compost). The volatile fatty acids utilized by SRB are generated by fermentative bacteria as a product of their metabolism (Sturman et al., 2008). The fermentative bacteria utilize simple organic compounds obtained by enzymatic hydrolysis of detrital matter composed of cellulose, hemicellulose, proteins, lipids, waxes, lignin (Reddy and DeLaune, 2008).

5.4.3 Carbon source for sulphate-reducing bacteria

The source of organic matter is a key requirement for the treatment of sulphate-containing wastewater by biological sulphate reduction. Carbon-deficiency is typical for some sulphate-rich wastewaters e.g. acid mine drainage or electroplating wastewater. Electron donors or appropriate carbon source should be added to such wastewaters to ensure reduction of sulphate and indirectly precipitation of metal sulphides if applicable (Liamleam and Annachhatre, 2007). Carbon source for SRB can be of internal or external origin. The latter can be added as aqueous solution to the wastewater and the former is present in the system as a solid form. Both types of carbon source can be used in CWs. Cheap, widely available and highly effective organic substrates are desirable in both cases.

Internal carbon source

Natural organic substrates tested to date include a wide range of roughage and organic wastes (Gibert et al., 2005). So far, a variety of compounds were tested as a carbon source for SRB, e.g. cow manure, rice stalks, straw, molasses, ryegrass, oak chips, spent oak from shiitake farms, spent mushroom compost, sewage sludge, organic-rich soil, activated sludge, compost of various origin, leaf mulch, mixtures of poultry manure, wood chips, and peat. Also more defined compounds such as polymers from lactic acid were investigated (Lens et al., 2007; Neculita et al., 2007). It is believed that high sulphate reduction rate can be achieved when a mixture of at least two materials is used. This is especially the case when relatively readily biodegradable material (e.g. manure) is mixed with more recalcitrant (e.g. saw dust) (Neculita et al., 2007) Some of these substrates may induce clogging, thus reducing treatment performance and leading

to system failure. Using a substrate with good hydraulic properties, such as gravel, and amending the wastewater with a soluble carbon source has therefore been suggested as an alternative (Machemer and Wildeman, 1992; Lesage, 2006).

External carbon source

Insufficient amount of organic carbon leads to incomplete biological reduction of sulphate. In this case electron donors or carbon sources must be added to enable sulphate reduction. Various types of solid organic substance, especially those with low molecular weight, have been used as internal carbon source as it was mentioned above. It is also possible to use synthetic carbon sources in liquid state such as acetate, butyrate, lactate, propionate and pyruvate. Ethanol and other alcohols can also be used as an internal carbon source. Most of these compounds can be produced in anaerobic bacterial degradation of detrital matter such as carbohydrates or proteins. Molasses was also reported to be used as an external carbon source due to high content of sucrose (Liamleam and Annachhatre, 2007). These soluble sources can be pure solutions of lactate, acetate or alcohols or waste products containing these substances, e.g. dairy wastewater.

SRB compete with methane producing bacteria for electron donors which are similar for both groups of bacteria (Song et al., 1998). The advantage in this competition depends, amongst others, on the amount of carbon available for bacteria and the ratio between electron donors and electron acceptors, expressed for example as COD/SO_4^{2-}. This ratio is adjusted according to the goals of the process: stimulation of methane production or production of sulphides, which could be predominant mechanism for precipitation of metals (as insoluble sulphides) from wastewater. The stoichiometry of sulphate reduction is given by:

$$8e + 8H^+ + SO_4^{2-} \rightarrow S^{2-} + 4H_2O \qquad (5.15)$$

Electron-accepting capacity of sulphate is twice that of O_2, thus theoretical minimum COD/SO_4^{2-} ratio required for achieving sulphate reduction is 0.67.

The activity of SRB during the treatment of raw electroplating wastewater treatment was studied by Song et al. (1998) in lab-scale tests (120-mL serum bottles). These authors evaluated different COD/SO_4^{2-} mass ratios to find that the value of 0.33 corresponded to the greatest reduction of sulphate per carbon source utilized by SRB. When COD/SO_4^{2-} was 0.33 the ratio of reduced sulphate per utilized organic carbon was 4.8 mg SO_4^{2-}/mg TOC and gradually decreased when COD/SO_4^{2-} was increased to 1.21. In this same study lactate was evaluated as the most efficient

electron donor for sulphate reduction. Sulphate degradation efficiency was between 70–75% when lactate was added, and 55–60% when acetate was used. Glucose was found to be the least efficient carbon source for SRB (45–50%). All these efficiencies were observed after 9 days of experiment. The disadvantage of using complex organic substrates such as lactate (and e.g. molasses) is that they are not fully oxidized by SRB, thus causing elevated COD levels in the effluent (Liamleam and Annachhatre, 2007). This statement is, however, arguable as lactate can serve a sole energy source for a group of SRB, and furthermore, lactate is oxidized to acetate, which can be easily utilized by other group of SRB and other bacteria, e.g. methanogens. Lactate is the most energetically advantageous substrate for SRB in terms of biomass produced and is superior to acetate, ethanol, propionate and others. The main drawback of using lactate is that only certain species of SRB can directly oxidize it to CO_2 (e.g. *Desulfotomaculum*), whereas other species can only partly oxidize it to acetate (e.g. *Desulfovibrio*) (Neculita et al., 2007).

6 Materials and methods

6.1 Experimental setup configuration and operational characteristics

Two experimental microcosm vertical-flow CWs were established in France and in Poland to study their feasibility to polish electroplating plant effluent. The CWs operated and investigated in France will be further denoted as System A, and System B will denote the CWs situated in Poland. Each system consisted of 12 columns (height, H = 80 cm, diameter, d = 20 cm) filled up to 63.5 cm with either mineral or mixed organic-mineral media. The active bed area (A) corresponding to these dimensions was 0.0314 m^2, the bed volume was 20 L. The schematic of column used in the experiment is shown in Fig. 6.1.

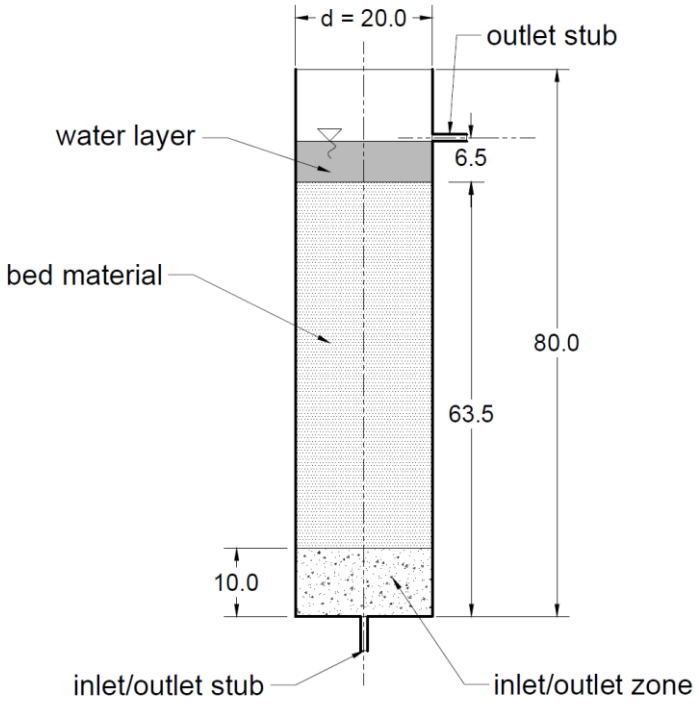


Figure 6.1 Cross section of a column used in the experiment (all dimensions in cm; in scale)

Six different configurations (each type was duplicated) were selected for the experiment based on feeding mode, type of bed media and the presence or absence of vegetation. The main objective of the system design was to promote metals (Cu, Ni, Pb, Zn) removal by precipitation as sulphides mediated by SRB, which, in general, are obligate anaerobes. Thus, most of the columns were designed to be strictly anaerobic (8 of 12 columns in each system). Anaerobic conditions in the columns were promoted by their construction and operation mode. These columns were operated in an upflow saturated mode with a 6.5-cm layer of water above the bed media preventing air from penetrating into the substrate. These columns, hereafter denoted as the UF columns, were fed from the bottom by peristaltic pump Masterflex® L/S® 7523-80 using

PharMed® BPT tubing (2.79 mm) and the outlet was situated 70 cm above the bottom. Thus, water depth (h) in the system was invariably 0.7 m and the corresponding nominal wetland volume was 22 L. Since, aerobic processes were also reported in the literature to be efficient for removal of metals and cyanides (e.g. Kadlec and Wallace, 2009) selected columns of the experimental system were operated as cycling anaerobic and aerobic (4 of 12 columns in the each system) which was enabled by the fill-and-drain (FaD) mode of operation. The FaD columns were fed manually from the top and drained from the bottom by opening a valve. A cycle in this type of batch columns consists of: filling phase, holding phase and draining phase. During draining phase air is sucked into the bed media thereby promoting aerobic transformation until onset of anaerobic conditions as soon as oxygen is depleted. The height of water layer above the bed media was the same as in the UF columns, this is, 6.5 cm, thus h was also 0.7 m. The cycling oxidizing and reducing phases occurring in the FaD columns are believed to have specific influences on most of the processes regulating the speciation, mobility and bioavailability of metals such as:

- sorption and desorption onto different solid components,
- adsorption and co-precipitation onto hydrous oxides of Fe and Mn,
- formation and decomposition of soluble and insoluble metal inorganic complex compounds,
- dissolution of carbonates, metal oxides and hydroxides,
- precipitation as sulphides under highly reducing conditions and their partial dissolution under oxic conditions.

It can be noticed that the processes occurring under oxic and anoxic conditions in single-step CW may be somewhat opposing, but it should be mentioned that the extended holding phase was expected to promote anoxic processes.

All the 12 columns were mounted on a steel rig as shown in photo (Fig. 6.2) for system A. The same rig in terms of construction was used in system B.

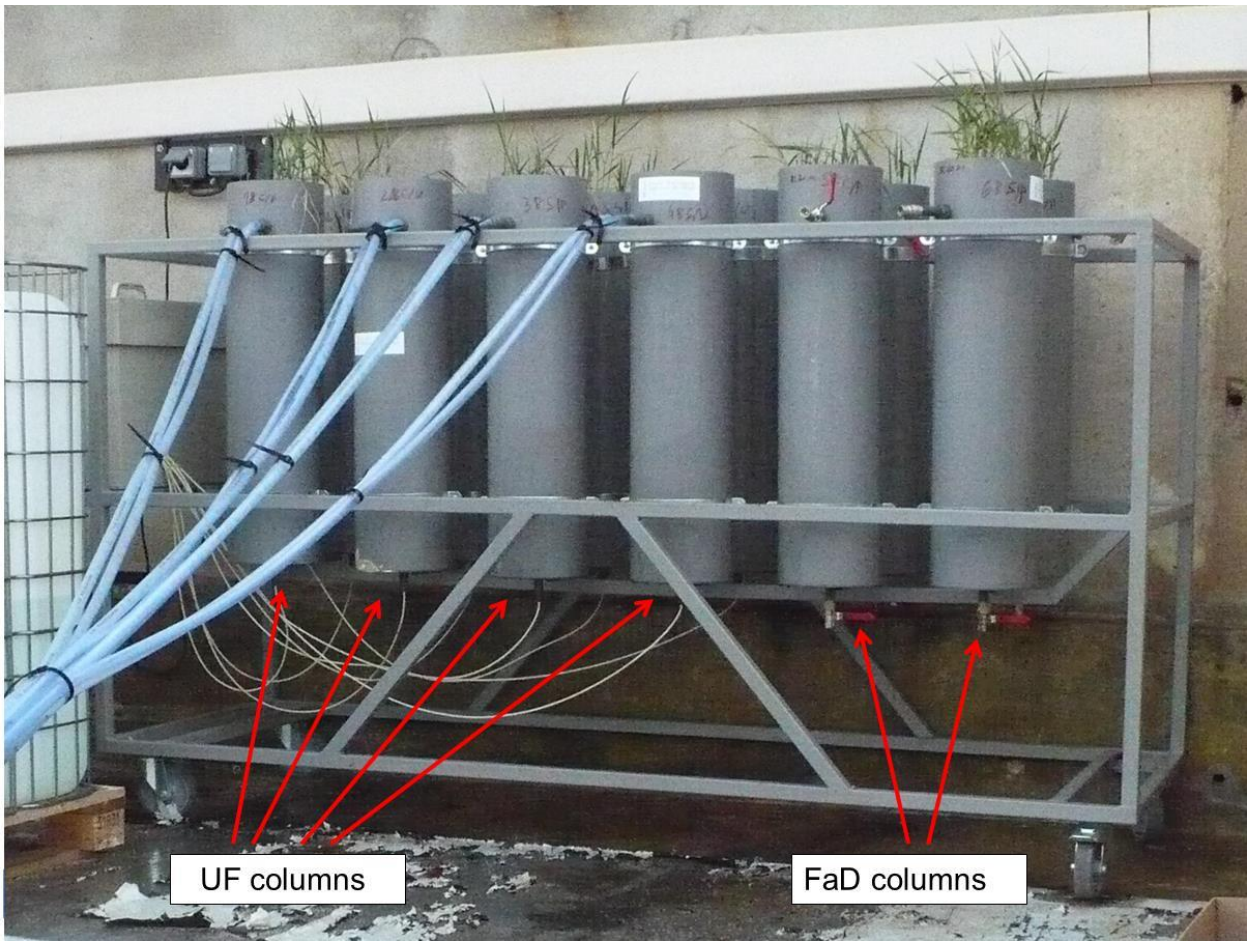


Figure 6.2 Experimental rig of system A with the UF and FaD columns indicated with arrows (week 2 of operation)

The construction and selection of filling materials was based on several studies reported in the literature in which similar microcosm systems were investigated. Ye (2006) used UF columns filled with sand, gravel and peat (and other materials) to treat neutral-pH acid mine drainages. The dimensions of those columns were 14 x 51 cm (d x H). Kassenga et al. (2003) used UF columns for the treatment of chlorinated volatile organic compounds. The size of columns used in that study was 15 x 60 cm (d x H) and were filled with sand and peat. Yadav et al. (2012) used PVC columns with dimensions 15 x 100 cm (d x H) for removal of metals. Lesage et al. (2007) used FaD columns (10-cm d) filled with gravel (3–8 mm) and straw up to 35 cm. Scholz et al. (2003) tested removal of Pb and Cu in a microcosm system using buckets (H = 58 cm) filled with various media and operated in DF mode. The type of bed media that were used in this experiment can be divided into mineral materials (sand and gravel) and organic materials (compost and peat). Importantly, the organic substrates were mixed with sand or gravel to avoid clogging. In system A two types of columns in terms of bed media were used: sand and compost-sand mixture, and in system B it was gravel and peat-gravel mixture. The mineral component in

the columns with organic media is omitted in this thesis for brevity. Thus, the columns with compost-sand mixture or peat-gravel mixture are referred to as ‘compost’ or ‘peat’ columns, respectively.

The initial operation parameters of the UF columns in systems A and B were based on the suggestions reported in the literature. In the PIRAMID Guidelines (2003) it was recommended that the hydraulic residence time (HRT) in the compost-based reactors for sulphate removal should be at least 40 h. Knowing the intended HRT it was possible to calculate corresponding flow rate based on formula (Kadlec and Wallace, 2009):

$$HRT = \frac{\varepsilon \cdot h \cdot A_{active}}{Q} \tag{6.1}$$

where: HRT is the hydraulic retention time, d; ε porosity, dimensionless; h is wetland water depth, m; A area of wetland containing water in active flow, m^2, Q is inlet flow rate, m^3/d.

The porosity of bed media was measured by draining the columns with organic-mineral and mineral media in the beginning and the end of the experiment. Hydraulic loading rate was calculated with formula:

$$q = \frac{Q}{A_{active}} \tag{6.2}$$

where q is the hydraulic loading rate, m/d.

The minimum and maximum values of porosity are given in Tab. 6.1. along with the other related parameters.

Table 6.1 Selected parameters of the experimental UF CWs (system B)

Porosity, ε	**Inlet flow rate, Q mL/min**	**HRT[1] d**	**Hydraulic loading rate, q m/d**
	2.00	2.7	0.09
0.35	3.25	1.6	0.15
	2.00	3.4	0.09
0.45	3.25	2.1	0.15

[1]hydraulic retention time

The porosity of the bed media as given in Tab. 6.1 cannot be regarded as the actual value as it was determined tentatively based on the drained volume of water from the columns regarded as the most representative. It should be also noticed that the porosity of the CW used in this experiment is not uniform as it is commonly assumed that the porosity of clean sand or gravel media is apt to be in the range 0.30–0.45 and the porosity of the water layer with or without

plants is much higher. These two porosities were lumped as one in the above calculations (Kadlec and Wallace, 2009).

It can be seen that the HRT in the UF columns at the inlet flow rate of 3.25 mL/min was in the range of 1.6–2.1 d depending on the type of column. It was observed that the porosity of the columns with organic-mineral media was slightly lower than for the columns with only mineral media. In general, this value of HRT was equal or slightly higher than the value suggested in the PIRAMID Guidelines (2003) this is at least 40 h (1.66 d). Two values of the inlet flow rate were applied in system B, which is discussed further in this thesis.

The FaD columns were operated in a different mode than the UF columns not only because of the varying feeding mode but also because of extended duration of the holding phase. In this experiment two durations of the holding phase were applied. The initial one in system B (and the only one in system A) was 14 d based on the microcosm study of Lesage et al. (2007) and then it was shortened to 7 d in system B.

Further implications of the operational parameters and the detailed information on how they were adjusted during the experiment are given in relevant chapters.

The treatment efficiency reported in this thesis, also referred to as removal efficiency or reduction, was calculated using formula:

$$\text{Removal} = \frac{C_{in} - C_{out}}{C_{in}} \tag{6.3}$$

where: removal is expressed in %, C_{in} is the influent concentration of contaminant, mg/L; and C_{out} is the effluent concentration of contaminant, mg/L.

6.1.1 Symbols of columns used in the experiment

The nomenclature used to denote the types of CWs used in the experiment will follow the nomenclature proposed by Fonder and Headley (2011), which is described in Section 4.1 of this thesis. The symbols for each type of a column operated during the experiment are presented with some of the corresponding key features in Fig. 6.3. Each type of column was duplicated. In each system there were 6 types and 12 columns. In total 24 columns were used in both systems.

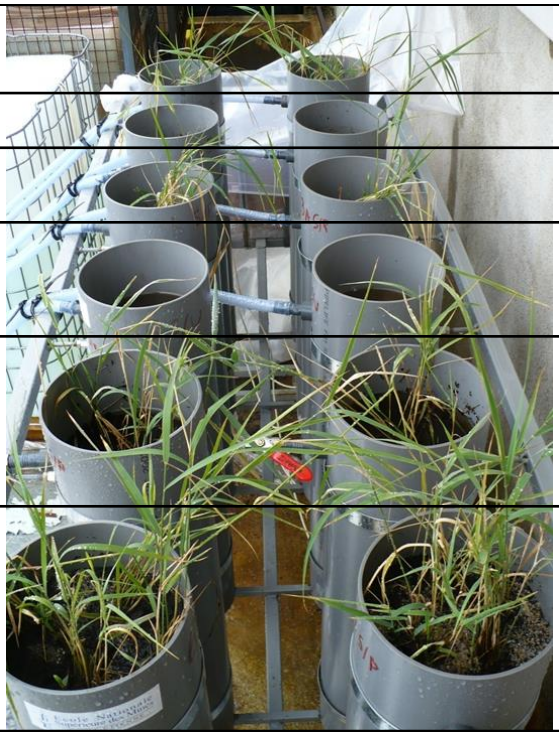
System A			System B	
Symbols	**Features**		**Symbols**	**Features**
UF-CP	compost & plants		UF-PP	peat & plants
UF-CU	compost		UF-PU	peat
UF-SP	sand & plants		UF-GP	gravel & plants
UF-SU	sand		UF-GU	gravel
FaD-CP	compost & plants		FaD-PP	peat & plants
FaD-SP	sand & plants		FaD-GP	gravel & plants

Figure 6.3 Configuration of the microcosm constructed wetlands in system A (left-hand columns) and system B (right-hand columns). Photo of system A two weeks after the start-up. System B was identical in terms of construction

The nomenclature system used in this thesis takes into account three easily identifiable physical traits of a CW, which allows distinction between the applied types of columns (also referred to as CWs). These three features are: flow direction and operation mode, type of bed media, and presence of plants. The flow direction or operation mode is indicated by the first part of a symbol (before hyphen):

- UF stands for upflow,
- FaD stands for fill-and-drain mode,

The type of bed media is indicated by the first letter after hyphen:

- C stand for compost (which in this experiment was mixed with sand),
- G stands for gravel,
- P stands for peat (which in this experiment was mixed with gravel),
- S stands for sand.

The second letter after hyphen indicates whether the column was planted (P) or unplanted (U). The other symbols that are used in this thesis are: UF denoting all the upflow CWs of system A

or B, FaD denoting all the fill-and-drain CWs of system A or B, UF-G denoting all the columns with gravel, either planted or unplanted, UF-C denoting all the columns with compost, either planted or unplanted, and FaD-C and FaD-G according to same naming scheme.

6.1.2 Configuration and operation of system A

System A was situated on the premises of the PEM electroplating plant in the town of Siaugues-Sainte-Marie, Auvergne, France, at an altitude of approx. 900 m above sea level. The system was operated in the outdoor conditions from June to October, 2011 and April to June, 2012. For the autumn-winter season the system was moved into the building of a WWTP of the company. The columns of this system were filled with silica quartz filtration sand (1–2.5 mm, more than 5% of free silica) or equivolume compost-sand mixture (hereafter referred to as 'compost'). The compost which was used was composed of: composted manure, algae and bark, and also brown and white peat. The other properties of the compost were: organic matter content 50% in DW, conductivity 45 mS/m, pH 6.8, water-holding capacity 700 mL/L. The application of compost as a bed medium of the CWs was previously reported in the literature (Section 5.4.3). The bed media were inoculated with SRB by adding cattle manure obtained from a local agricultural farm. Selected columns were planted with potted seedlings (3 per column) of *Phragmites australis* (Cav.) Trin. ex Steud (Common reed).

Operation disturbances occurred in system A in the period from October, 2011 to April, 2012 when the UF columns where fed discontinuously due to pumping system's failure. For this reason the performance results of system A are presented and discussed only for the batch columns (FaD-CP and FaD-SP). The system was fed with real wastewater originating from the PEM plant and containing Al, B, Cu, Ni, Fe, Pb, Zn, cyanides and sulphates as the contaminants of concern. The system was fed with wastewater without cyanides and cyanide wastewater from one-cubic-metre containers. The former was the final effluent from the wastewater treatment plant of the electroplating plant, and the latter was the pretreated wastewater after metal precipitation stage taken from the acidic buffer tank (Fig. 3.1). The feed without cyanides was fed from June, 2011 to June, 2012, and the cyanide wastewater was fed during May and June, 2012 to the columns which were previously fed with the feed without cyanides. In May and June, 2012 columns were fed in parallel by both types of influent, therefore they were not replicated. The inflow rate was 3.25 mL/min for the UF columns (when in operation) and the batch duration was 14 d (in the FaD columns), during the whole experiment. The operation chart for system A is presented in Tab. 6.2.

Table 6.2 Operation chart for system A

Operation mode	**Columns**	**Weeks of operation**		
		1−15	16−45	45−56
		Month and year		
		June 11−Sept 11	Sept 11−Apr 12	Apr 12−June 12
Influent quantity				
Q_{in} = 3.25 mL/min	UF			
Discontinuous feeding[1]	UF			
14-day batch duration	FaD			
Influent quality				
Wastewater without cyanides	All			
Cyanide wastewater	All[2]			

[1]due to pump failure, [2]columns fed in parallel, no replication

6.1.3 Configuration and operation of system B

System B was situated in laboratory conditions in Gliwice, Poland. It was in operation from January, 2012 to January, 2013, which is 53 weeks. The columns of system B were filled with quartz gravel (3–8 mm) or equivolume gravel-peat mixture (hereafter referred to as 'peat'). The pH value of the peat was 3.5–4.5; organic matter content 89.9% of DW, and water-holding capacity 746 mL/L. The application of peat as a bed medium of the CWs was previously reported in the literature (Section 5.4.3). The bed media were inoculated with SRB using anaerobic sludge (mesophilic anaerobic digestion) with initial 14-day batch of wastewater to all the columns prior to the start-up of the experiment. Selected columns were planted with rhizomes of *Phragmites australis* (Cav.) Trin. ex Steud. After a few months of the experiment it was observed that also *Phalaris arundinacea* L. (Reed canary grass) was present in the planted columns creating a mix stand with *P. australis. Phalaris arundinacea* was mistakenly shipped with *P. australis* by vendor.

The feed of system B was prepared by dissolving salts of metals in tap water in a 250-L container. The salts used were $Pb(NO_3)_2$, $NiSO_4 \cdot 6H_2O$, $ZnCl_2$, $CuSO_4 \cdot 5H_2O$, Na_2SO_4. System B was fed with low-strength and high-strength wastewater. The former was fed from January, 2012 to April, 2012, and the latter from May, 2012 to January, 2013. The concentration of sulphates was equal in the both influents at 500 mg/L (resulting from Na_2SO_4), and the Pb concentration was also equal at 1 mg/L. Concentration of Cu, Ni and Zn was raised from 1 mg/L in low-strength to 5 mg/L in high-strength influent in week 18 of the experiment. In week 33 the inflow rate to the UF columns was decreased from 3.25 to 2.00 mL/min. The batch duration in the FaD columns was shortened in week 33 from 14 d to 7 d. In week 44 of the experiment lactates were started to be added to the influent fed to the columns with gravel (UF-GP, UF-GU, FaD, all in duplicates as for the other types of the feed). In week 48 cyanide was started to be added to all the types of columns. Cyanide was added as KCN in a dosage equal to 2.0 mg/L of total cyanide.

In weeks 50−51 the FaD columns were fed with an influent simulating the raw wastewater based on the data obtained from the PEM company. This experiment is further referred to as the 'emergency event'. All the operation periods are given in Tab. 6.3.

Table 6.3 Operation chart for system B

Operation mode	**Columns**	**Weeks of operation**						
		1−17	18−32	33−43	44−47	48−49	50−51	52−53
		Month and year						
		Jan 12 May 12	May 12 Aug 12	Aug 12 Nov 12	Nov12 Dec 12	Dec 12 Dec 12	Dec 12 Jan 13	Jan 13 Jan 13
Influent quantity								
Q_{in} = 3.25 mL/min	UF							
14-day batch duration	FaD							
Q_{in} = 2.00 mL/min	UF							
7-day batch duration	FaD							
Influent quality								
Low-Strength	All							
High-Strength	UF							
	FaD							
Addition of lactate	UF-P							
	UF-G							
	FaD-P							
	FaD-G							
Addition of cyanide	All							
Emergency event	FaD							

Lactates were added to the feed as 80% lactic acid and the dosage was calculated based on the assumption that a target influent ratio COD/SO_4^{2-} is 0.3. This value was based on the study of Song et al. (1998) where it was found to be the optimum ratio. The concentration of sulphates used for calculation was 800 mg/L and the corresponding COD of lactates was 240 mg O_2/L. The concentration of SO_4^{2-} was invariably higher than the added 500 mg/L, as SO_4^{2-} present in the tap water also contributed to the total concentration of SO_4^{2-}. It was assumed that COD of the influent before the addition of lactates will not be taken into account while calculating the dosage of lactates. The dose of lactates was calculated knowing the amount of COD required and the stoichiometry of sulphate reduction when lactate is the electron donor (eq. 6.4).

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2CO_2 + 2H_2O + S^{2-} \quad (6.4)$$

When lactate is used as an electron donor for sulphate reduction stoichiometrically 2 moles of lactate are needed per 1 mole of SO_4^{2-} (eq. 6.5). One mole of lactate corresponds to 96 g of COD (3 moles of O_2), which is based on this stoichiometry:

$$CH_3CHOHCOOH + 3O_2 \rightarrow 3CO_2 + 3H_2O \quad (6.5)$$

The calculated dosage of lactates was 225 mg lactic acid per 1L of wastewater (0.234 mL of 80% lactic acid per 1L of wastewater, density 1.2 g/mL).

6.2 Physicochemical analysis of wastewater

6.2.1 Sampling

Water was sampled as follows: (i) from the UF columns: samples were collected by either syringe from outlet zones of columns or collected to plastic bottles connected by plastic hose to the outflow stubs of columns; (ii) from the FaD columns: prior to sampling columns were completely drained by opening a valve at the bottom of each column; effluent was collected into bucket, thoroughly mixed avoiding aeration and then samples were withdrawn; (iii) from the feed container: samples taken by syringe or by immersion of sample vials, depending on water level.

6.2.2 Analysis of metals, boron and sulphur

In system A metals (Al, Cu, Fe, Mn, Ni, Zn), B and S were analysed with inductively coupled plasma atomic emission spectrometry (ICP-AES; Horiba Jobin-Yvon JY138 Ultrace apparatus) after acidifying filtered (0.22 μm) samples with 65% HNO_3 to pH < 2. The detection limits for metal analysis by ICP-AES were 0.001, 0.01, 0.001, 0.002, 0.001, 0.002, 0.015, 0.2, 0.001 mg/ L for Al, B, Cu, Fe, Mn, Ni, Pb, S, Zn.

In system B metals (Cu, Fe, Mn, Ni, Pb, Zn) were analysed with flame atomic adsorption spectrometry (FAAS; Varian Spectra AA 800) after acidifying filtered samples with 65% HNO_3 to pH < 2. The detection limits for metal analysis by FAAS were 0.0015, 0.0025, 0.0010, 0.0040, 0.0020, 0.0010 mg/L for Cu, Fe, Mn, Ni, Pb, Zn, respectively.

6.2.3 Analysis of cyanide

For the samples taken from system A the concentration of free cyanide was determined photometrically at 588 nm with Hach Lange cuvette test LCK 315 (measuring range 0.01–0.60 mg/L CN^-) using Hach-Lange DR2800 spectrophotometer. In this method cyanides react with chlorine to form cyanogen chloride, which in turn reacts with pyridine in the presence of barbituric acid, condensing to form a violet colorant. This method covers the determination of HCN and of CN complexes that are amenable to chlorination and also thiocynates according to the APHA Standard Methods (method 4500-CN-H; Eaton et al., 2005).

For the samples taken from system B readily liberated cyanides were determined using Merck Cuvette test 1.09701.0001. In this method (for samples decomposed by digestion) cyanide ions react with a chlorinating agent to form cyanogen chloride, which in turn reacts with 1,3-dimethylbarbituric acid to form a violet dye (pyridine-free König reaction). This dye was determined photometrically using Merck Spectroquant NOVA 400 spectrophotometer. The method is analogous to EPA 335.2, ISO 6703-2:1984, ISO 6703-3:1984 and DIN 38405 D13.

6.2.4 Chemical oxygen demand and total organic carbon analysis

For samples taken from system A chemical oxygen demand (COD) was determined by Hach Lange cuvette test LCI 500 according to ISO method 15705 (2002) using Hach-Lange DR2800 spectrophotometer. For samples taken from system B COD of wastewater was determined using standard method of reflux digestion and titration (ISO 6060:1989).

For samples taken from system B total carbon, inorganic carbon and total organic carbon (TOC) were determined by TOC analyser Shimadzu TOC – VCSH in weeks 1-17 (ceased after equipment failure).

6.2.5 Sulphate analysis

For the samples taken from system B SO_4^{2-} were determined using Merck Cuvette test 1.14791.0001. In this method (for samples decomposed by digestion) sulphate ions react with barium iodate, liberating iodate ions in the process. These oxidize tannin to a brown-red compound that was determined photometrically using Merck Spectroquant NOVA 400 spectrophotometer.

6.2.6 pH and oxidation-reduction potential measurements

Oxidation-reduction potential (ORP) was measured (with a redox electrode) in the water samples from the bottom, top and water layer of the columns directly after withdrawal. pH was measured in the effluent and effluent of both system using an electrode connected to a pH-meter WTW 330.

6.3 Sequential extraction procedure (system A)

The manner in which metals (and S) were bound to substrate in the experimental system was assessed by the SEP based on the BCR2 protocol (Rauret et al., 1999). This procedure consists of three steps devised to extract three operationally defined fractions. In the first step exchangeable, water- and acid-soluble fraction was targeted and 0.11 M acetic acid was used. The second step

was to extract reducible phase by the use of 0.5 M hydroxylammonium hydrochloride at pH 1.5. The target phase in step 3 was reducible fraction and reagents used were 8.8 M H_2O_2 at 85°C and then 1 M ammonium acetate at pH 2.0. As an internal check on the procedure, the residue from step 3 was digested in *aqua regia* (step 4) and the amount of metals extracted (i.e., sum of step 1, step 2, step 3 and residue) was compared with that obtained by independent *aqua regia* digestion of a separate sample of the sediment. The SEP was conducted with approx. 1 g of substrate accurately weighed in 50 mL polypropylene centrifuge tubes, to which extracting solutions were added in a volume twice as small as in the BCR2 protocol. Substrate samples were taken from the bottom and top of a column. It was assumed that that these zone were represented by 150 mm layer from the bottom or top surface. Each step (1–3) involved shaking the samples with extracting solution for 16 h on the end-over-end shaker. The extract from the solid residue was separated by centrifugation at 3000 g for 20 min and the supernatant was decanted into a PE container, filtered and stored at 4°C for ICP-AES analyses (Horiba Jobin-Yvon JY138 Ultrace apparatus). The residue was washed by adding 20 mL of distilled water, shaking for 15 min, centrifuging for 20 min, and then discarding the supernatant. Reaction with H_2O_2 in step 3, and *aqua regia* digestion were performed in 30 mL PTFE vials. The *aqua regia* digestion was based on the protocols of ISO 11466 (1995) and Sastre et al. (2002). In this procedure 1 g of sample was weighed into 30 mL PTFE vials and 12 mL of *aqua regia* was added (9 mL of 37% HCl and 3 mL of 65% HNO_3) and digested at room temperature for 16 h. Afterwards the suspension was digested at 130 °C for 2 h in closed vials. The obtained suspension was filtered through an ashless filter, and then diluted to 32 mL with distilled water, and stored in PE bottles at 4°C for analyses. The amount of metals extracted (and S) in the independent *aqua regia* digestion was assumed to represent pseudo-total concentration of metals in substrate. For the evaluation of measurement precision and accuracy the certified reference material (soil) NCS ZC73006 (China National Analysis Centre for Iron and Steel) was used. The reproducibility expressed as a relative standard deviation of the quality control samples were less than 10% (n = 7). Average recoveries between analysed and certified reference material values, for step 1-4 of the BCR protocol, were (n = 8): Al 39.2%, Cu 65.52%, Fe 57.5%, Mn 83.4%, Ni 65.7%, S 137.4%, Zn 67.4%. The recoveries between the sum of steps 1–4 and single-step *aqua regia* digestion of substrate samples were (n = 27): Al 82.12%, Cu 105.41%, Fe 70.19%, Mn 94.10%, Ni 108.82%, S 85.87%, Zn 180.46%.

The low recovery for some of the elements in comparison with the certified reference material is not unusual. *Aqua regia* digestion, which is recommended in the BCR2 protocol to extract the residual fraction, is not sufficient to achieve complete dissolution of siliceous and refractory

materials (Larner et al., 2006). Recoveries as low as 39% for Al and 57% for Cr were obtained in the *aqua regia* digestion of a certified reference material by Larner et al. (2006). The discrepancies between the amount of metals determined by steps 1–4 of the BCR protocol and the independent *aqua regia* digestion may result from the method but also from the properties of the sample. The former aspect is widely discussed in the literature (Filgueiras et al., 2002; Bacon and Davidson, 2008), whereas the latter may be related to poor homogeneity of the samples and the presence of fine organic debris that could be lost from the extracting solution. Li et al. (2010) found recoveries between 87–124% for Zn, Cu, Pb and Cd to be indicating 'good agreement' between the sum of steps 1–4 and the independent *aqua regia* digestion. Hang et al. (2009) extracted metals from river sediments with recoveries between 82–119%.

6.4 Sequential extraction procedure (system B)

The BCR2 protocol (Rauret et al., 1999) was also employed for analysis of bed media sampled from system B. Only the differences from the procedure described in section 6.3 will be given. Instead of 1 g approx. 2 g of substrate was taken for analysis, which was performed in 150-mL vials. The increased mass of samples was taken because of their poor homogeneity due to the presence of coarse gravel in contrast to sand used in system A. The samples were air-dried before analysis for 48 hours. The extracting solutions were added in a volume twice as large as in the BCR2 protocol, with the exception of step 3. The *aqua regia* digestion was based on the PN-ISO 11466:2002 norm using 50 mL of the extracting solution. The extracts were analysed for Cu, Ni, Pb, Zn using FAAS (AA Scan 1 Thermo Jarrell Ash apparatus). The recoveries between the sum of steps 1-4 and single-step *aqua regia* digestion of substrate samples were: Cu 109.56%, Ni 129.92%, Pb 123.75%, Zn 101.84%.

6.5 Plant analysis

Biomass of *Phragmites australis* growing in the experimental columns of system A were collected in May, 2012 (week 48) and divided into above- and belowground parts. The former were clipped just above the bed surface and the latter were dug out with the adhering soil down to the 10 cm below the surface. The plants were washed with deionised water, ground, dried at 40°C until constant weight, weighted (500 mg DW) into clean, dry PTFE screw cap digestion tubes and mineralized (according to the in-house digestion protocol). In the applied digestion procedure plant material was treated with concentrated nitric acid (65% HNO_3, analytical grade) for 1 h at 50°C then 12 h at room temperature. Three mL of hydrogen peroxide (36.5% H_2O_2, analytical grade) were added and samples were digested for 0.5 h with untwisted cap and then

heated at 70°C until approx. 1 mL remained. Then *aqua regia* was added and samples were digested for 2h at 150°C. *Aqua regia* was prepared by adding 4.5 mL HCl (37%, analytical grade) and 1.5 mL 65% HNO_3 to each tube. Samples were then cooled, tubes were opened (recovering condensates from the cap), and kept at 70°C until complete evaporation. Dry samples were finally resuspended in 10 ml HCl (2 M) by heating for 1 h at 100°C in closed tubes. Mineralized samples were cooled, filtered and analysed using ICP-AES (Horiba Jobin-Yvon JY138 Ultrace apparatus). For all the measurements presented, standard quality control was performed. Quality control samples consisted of triplicate samples. For the evaluation of measurement precision and accuracy the certified reference material CTA-OTL-1 Oriental Tobacco Leaves (Institute of Nuclear Chemistry and Technology, Warsaw, Poland) was used. The reproducibility expressed as a relative standard deviation of the quality control samples were less than 4%. Average recoveries (n = 4) between analysed and certified reference material values were: Al 42.0%, Cu 97.8%, Fe 72.8%, Mn 82.3%, Ni 41.6%, S 90.8%, Zn 86.9%. Low recoveries may be results of partial dissolution or volatilization (DuLaing et al, 2003). For example, recoveries of Ni and Cr were found to be only 29–58% by various methods including HNO3 and H_2O_2 (without *aqua regia*) in the comparative study reported by DuLaing et al. (2003). Only microwave-assisted destruction method ensured acceptable recoveries for Ni and Cr and this method yielded the best recoveries (DuLaing et al, 2003).

6.6 Adsorption assay

Sorption tests were based on the protocol of Lesage (2006). In this procedure raw bed media (of system A) adsorption capacity for metals and boron was tested. The amount of material depended on its density and was: 0.5 g for compost, 0.614 g for mixture of compost and sand (50/50% by volume), and 25 g for sand. The volume of the solution containing metals and B was 50 mL for compost and its mixture with sand, and 45 mL for sand. The composition of the solutions was similar as for the microcosm experiment and was added with increasing levels of Al, B, Cu, Fe, Ni and Zn: 1, 5, 10, 50 and 100 mg/L. Metals were spiked from a stock solution that contained 1000 mg/L of each metal and B. Sorption tests were performed at room temperature with no pH adjustment. 50-mL vials were placed on an end-over-end shaker for 2 h after which the solution was separated from the media and analysed for metals and B after acidification with 65% HNO_3. The concentration adsorbed onto the surface of the tested media (q_s, mg/kg DW) was calculated based on the difference between initial and equilibrium metal (or B) concentrations in the solution. The equilibrium concentration in the solution (C_e, mg/L) and the sorbed concentration qs were fitted to the Langmuir model:

$$q_s = X_m \frac{b \cdot C_e}{1 + b \cdot C_e} \tag{6.6}$$

where X_m (mg/kg DW) is the maximum sorption capacity and b (L/mg) is a measure of sorption energy. Control experiments were performed to measure sorption onto the glassware and filter paper. For Co, Ni and Zn sorption was negligible and accounted for only 1.5, 1.6 and 3.3% of the initial metal concentration. However, the initial Cu concentration was reduced by 9% by a combination of sorption on the glassware and filter paper. Data were corrected for these sorption effects. Metals and B were analysed by means of ICP-AES using a Horiba Jobin-Yvon JY138 Ultrace apparatus. Parameter estimates of Langmuir isotherms were obtained via nonlinear least-squares regression using the *nlinfit* function of MATLAB (R2010a) Software (MATLAB 10).

6.7 SEM-EDS analysis

Surface morphology of the samples was performed using a scanning electron microscope (SEM) HITACHI S-3400N with backscattered electron detector (BSE). The analysis of chemical composition of the samples was performed using energy-dispersive X-ray spectroscopy (EDS) Thermo Noran System (System Six). EDS analysis is a semi-quantitative method and the limit of detection is ca. 0.2 atomic % depending on element. SEM imaging and chemical microanalysis were performed in a low vacuum (50 Pa) and at accelerating voltage of 15 kV. Similarly to the procedure for SEP substrate samples were taken from the bottom and top of a column. It was assumed that that these zone were represented by 150 mm layer from the bottom or top surface. Only the samples of gravel from the UF-GU columns were taken from the topmost layer of the bed medium. The samples analysed by SEM-EDS were subdivided from the samples taken for the SEP analysis and then placed on titanium sample mounts covered with carbon tape. The additional samples were: filters through which wastewater samples from columns UF-GU and UF-PP (system B) were filtered, and gravel taken from the outlet stub of column FaD-PP (system B). To this end 1.5 L wastewater was withdrawn from the water layer of the UF-PP and UF-GU columns and was filtered through a 0.45 μm filter.

6.8 Statistical analysis

Most of the subsets of the performance data had non-normal distribution, therefore non-parametric statistical test were used to compare these subsets. Median and the median absolute deviation (MAD) were used as descriptors of the central tendency and dispersion of data distributions for non-normally distributed data (especially, wastewater quality). Arithmetic mean and standard deviation (SD) were used for normally-distributed data (esp. metal concentration in

plants and bed media). The Shapiro-Wilk W test was employed to test for normality of the data. Dependent groups were analysed using the Wilcoxon matched pair test and independent groups were analysed using the Mann-Whitney U test. Normally distributed data were analysed by the use of Student's t-test. Differences were considered statistically significant if $p < 0.05$. Statistical testing was performed using the STATISTICA 10 software (StatSoft, Inc., 2011).

7 Results and discussion

The results presented and discussed in this chapter regard two main aspects of the experiment. The first aspect is the treatment efficiency of both experimental systems and effects of operational conditions and intrinsic properties of the systems. The other aspect is related to the components of the experimental CWs, chiefly plants and bed media, and how they influenced the treatment processes occurring in the CWs studied.

7.1 Treatment efficiency of constructed wetlands in system A

Influent and effluent quality and the corresponding treatment efficiency in the FaD columns of system A are presented in Table 7.1 and 7.2, for the feed without cyanides and cyanide wastewater, respectively. System A was fed with real electroplating wastewater from the electroplating plant in Siaugues-Sainte-Marie, France. Both types of influents had similar qualitative composition with the exception of cyanides, which were present only in the cyanide wastewater. The other contaminants were metals: Al, Cu, Ni, Zn, Fe and Mn; B, S and organics measured as COD.

The feed without cyanides was fed to the system A throughout all of the experiment and it was the treated effluent that was discharged into a stream. Concentration of B, S, Mn, Ni and Zn was higher in this type of wastewater comparing to the cyanide wastewater.

Table 7.1 Overall inlet and effluent concentrations (median ± MAD, mg/L) and removal efficiencies (median ± MAD, %) of contaminants in the FaD columns of system A fed with the feed without cyanides, (n = 6), weeks 1−56

Parameter	Influent	Effluent from FaD-CP CW		Effluent from FaD-SP CW	
		Concentration	Removal[1]	Concentration	Removal[1]
Al	0.264±0.638	0.063±0.040	79.1±10.7	0.064±0.048	84.4±13.5
Cu	0.083±0.016	0.003±0.002	97.2±1.4	0.001±0.001	98.9±0.3
Fe	0.012±0.007	0.258±0.170	-1912.5±1954.1	0.648±0.531	-8117.1±7999.5
Mn	0.003±0.001	0.848±0.125	-19842.8±5469.3	0.199±0.044	-7017.2±4114.6
Ni	0.058±0.013	0.013±0.004	77.9±7.4	0.013±0.004	80.3±5.4
Zn	0.022±0.015	0.054±0.030	-167.6±89.3	0.046±0.015	-296.0±277.0
B	5.43±0.14	3.47±0.46	37.7±28.5	5.34±0.15	1.4±11.0
COD	38.0	108.7	-186.6	84.6	-123.1
S	538.9±4.7	375.9±85.4	18.3±3.4	515.7±43.1	4.3±8.9
pH	8.5	6.74		6.59	

[1]negative values indicate release of a given element

The feed with cyanides influent was characterized by elevated concentration of Al, Cu, and detectable amounts of cyanide. This influent was the pretreated wastewater taken from a metal-precipitation step of the treatment in the WWTP of the electroplating plant (Section 3.3).

Table 7.2 Overall influent and effluent concentration (median ± MAD, mg/L) and reduction of contaminants in system A (median ± MAD, %) fed with cyanide wastewater, (n = 4), weeks 45−56

Parameter	Influent	Effluent from FaD-CP CW		Effluent from FaD-SP CW	
		Concentration	Removal[2]	Concentration	Removal[2]
Al	2.768±0.111	0.242±0.094	91.4±3.0	0.270±0.097	90.4±3.6
Cu	2.315±0.251	0.003±0.003	99.9±0.1	0.005±0.003	99.9±0.1
Fe	0.033±0.001	0.059±0.008	-140.8±76.5	0.270±0.026	-724.6±73.6
Mn	0.002±0.001	0.439±0.010	-24008.5±2053.0	0.052±0.004	-2547.2±486.1
Ni	0.037±0.012	0.032±0.006	28.2±17.3	0.044±0.004	-47.0±55.4
Zn	0.015±0.009	0.040±0.004	-304.2±320.4	0.032±0.023	-332.3±394.4
B	4.03±0.03	2.21±0.38	46.8±7.4	4.36±0.50	-1.9±13.5
CN[1]	1.835±0.090	0.255±0.059	86.0±4.0	0.246±0.033	86.9±2.6
COD	40.2	244.5	-488.3	145.5	-299.3
S	393.9±0.1	293.5±30.6	21.4±7.6	350.0±10.9	7.9±3.4
pH	9.05	6.88		7.22	

[1]free cyanide; [2]negative values indicate release of a given element

The removal efficiency for metals was in the decreasing order of Cu > Al > Ni. The removal of Al and Cu was invariably very high in system A, over 79% and 90%, respectively. The removal efficiency for Al and Cu was higher in the columns fed with cyanide wastewater (statistically different). In contrast, the removal of Ni was lower in the columns fed with cyanide wastewater. Ni was even released from the FaD-SP column fed with this influent in the last 12 weeks of the experiment. Iron, Mn and Zn were released from both types of the FaD columns. Interestingly, the Ni concentration was lower in the cyanide wastewater, however, not statistically different from the feed without cyanides. Thus, it can be assumed that the decreased removal of Ni can be attributed to the effect of the other pollutants present in the cyanide wastewater in higher concentrations, namely Al, Cu and cyanide, or pH of this influent. System A was also very efficient in terms of cyanide removal, which was ≥ 86% in both FaD columns fed with the cyanide wastewater. Statistically significant differences between the treatment efficiency in the FaD-CP and FaD-SP columns were observed for B, S and Mn for both types of influent. Both reduction of B and S was higher in the columns filled with compost. The removal of B was markedly higher in FaD-CP columns for both types of wastewater in system A. Enhanced removal of B in the FaD-CP columns may be attributed to its sorption to organic matter within the bed of the columns. Higher removal efficiency of B was recorded for the FaD-CP columns fed with cyanide wastewater, in which B concentration was lower by 1.4 mg/L. The FaD-SP columns were inefficient in the removal of B with almost no reduction or slight release. The removal of S was much higher in the FaD-CP columns, which might be related to the stimulative effect of internal carbon source on SRB. This, however, had no effect on the removal of metals. It was observed that the COD value of the treated wastewater increased by 1- to 2-fold. This was caused by the presence of manure in the FaD-SP CWs and both manure and compost in the FaD-

CP CWs, in which the release was higher. The release of Mn was higher in the FaD-CP columns by an order of magnitude. This might be related to the fact that the Mn content in sand and compost was 4.834 and 42.797 mg/kg DW, respectively (Section 7.7.1). Apart from Mn also Fe and Zn were released from both FaD columns. The release of Mn was higher for the FaD-CP column and Fe for FaD-SP column and was ten- to hundred-fold higher as compared to the influent concentration. The release of Fe and Mn can probably attributed to dissolution of Fe and Mn oxides and hydroxides, which is promoted under anaerobic conditions. In contrast to Fe and Mn, the release of Zn was only 1- to 3-fold higher than the influent concentration. The provenance of Zn is probably different, as Fe and Mn were probably present in the raw bed media, and Zn was mainly desorbed after the sorption capacity of the media was exhausted. The composition of raw bed media was analysed and is presented and discussed in Section 7.7.1.

As shown in Tab. 7.1 and 7.2 it is noteworthy that electroplating wastewater is a poor source of external carbon for stimulation of the SRB activity. COD as a lumped parameter does not determine whether organic matter could be utilized by heterotrophic bacteria. Speciation analyses were not performed but it is likely that organic matter in the real electroplating wastewater could not be readily available for bacteria. The COD of the wastewater could be related to residual organic auxiliary compounds used in electroplating, which can be slowly biodegradable or biorecalcitrant. Considering the potential for bacterial sulphate reduction it should be mentioned that S concentration was high enough for this process to occur, thus carbon was the limiting substrate. The speciation of sulphur was not performed routinely, but in the beginning of the experiment the concentration of sulphate was measured several times and was approx. 1.5 g/L. This value was also reported by a plant's operator as a typical value for the final effluent.

On comparing the concentration of individual elements (or COD) in the feed without cyanides (Tab. 7.1) and the cyanide wastewater (Tab. 7.2) it can be seen that the latter was not always of laden with higher concentrations of metals and other compounds. For example, the concentration of sulphates is higher in the feed without cyanides because sulphuric acid was used to adjust the pH of the final effluent. In the case of the other elements the higher concentration in the feed without cyanides may simply stem from the fact that both feeds were stored in one-cubic-meter tanks refilled with a certain time shift relative to each other. Thus, both types of the feed originated from different periods of the treatment in the WWTP.

7.2 Treatment efficiency of constructed wetlands in system B

The data collected from system B can be subjected to more detailed analysis than the data from system A. This is because more data points are available for system B and because the control and monitoring of system B was more efficient. Influent and effluent concentrations were significantly different for all the metals in all types of column as assessed statistically with $p < 0.001$.

7.2.1 Overall treatment efficiency for metals

System B was used to treat synthetic electroplating wastewater in six types of columns, of which some were left unplanted. The unplanted columns were used as a reference for the planted systems and are not considered CWs in this thesis. The unplanted columns are not shown in Fig. 7.1 for this reason and for clarity.

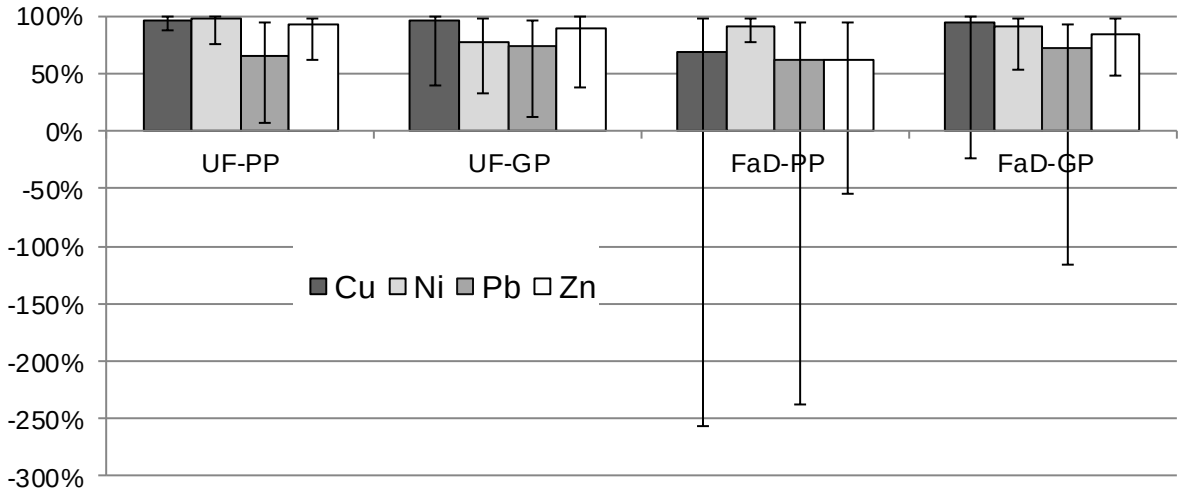


Figure 7.1 Median removal efficiencies (and max. and min. values indicated by whiskers) of the CWs in system B during the experiment (n = 24−26), weeks 1−53

It is debatable if showing averaged results for all of the experiment is meaningful, however, the results shown in Fig. 7.1 contain interesting information on system B and can be regarded as a starting point for a further discussion. The results presented in Fig. 7.1 do not allow to assume general order of removal efficiency for metals in all the CWs. The decreasing removal order in the UF-PP CW was Ni > Cu > Zn > Pb, in the UF-GP CW it was Cu > Zn > Ni > Pb, in the FaD-PP CW it was Ni > Cu > Zn > Pb, and in the FaD-GP CW the order was Cu > Ni > Zn > Pb. The lowest removal efficiency was observed for Pb in all the types of the investigated CWs. The CWs filled with organic media (UF-PP and FaD-PP) reduced the Ni concentration with higher efficiency than observed for Cu. Conversly, Cu was attenuated to a higher extent than Ni in the

CWs with only mineral media (UF-GP and FaD-GP). The differences between the removal efficiencies of Cu and Ni in the UF-PP and FaD-GP CWs were not pronounced: less than 1% and less than 2%, respectively. The other observation made for the results presented in Fig. 7.1 is that the UF CWs were more stable and reliable systems than the FaD CW as the ranges between minimum and maxium values for the UF CWs is not a broad as for the FaD CW. Only the removal of Ni in both FaD CWs was stable and the removal of Zn was stable in the FaD-GP column, this is, in these cases no release occurred. Additional observation that can be made is that the FaD-GP CWs provided more stable (or more accurately: less unstable) operation than FaD-PP. In contrast to the FaD CWs, the UF system that proved to be more stable was the one filled with organic medium (UF-PP) as compared to the system with only mineral bed (UF-GP).

7.2.2 Treatment efficiency of the UF-PP columns

The averaged data as shown in Fig. 7.1 facilitate comparison of several systems but does not allow visualizing the time course of the process. Figures 7.2−7.5 present the influent and effluent concentrations of Cu, Ni, Pb and Zn, respectively. These graphs allow for observation of time-dependent changes due to internal factors and the changes introduced intentionally (or sometimes occurring stochastically) during the experiment (external factors). The internal factors that govern the treatment in the studied CWs can be sorption capacity for metals, stimulation or inhibition of microorganisms, clogging, etc. The external factors can be controlled and monitored (e.g. flow rate, feed composition), monitored but non-controlled (e.g. temperature) and non-controlled and non-monitored. The internal behaviour of the system can be to some extent caused by the external factors controlled during the experiment. For example, the activity of SRB can be increased providing more advantageous conditions for their growth.

It is particularly interesting to refer to the operating chart of system B (Tab. 6.3) to observe the system’s response to a given operation mode.

Figure 7.2 presents the influent and effluent concentrations of Cu for the UF-PP CWs during the whole experiment. The trendlines represent two-period moving averages.

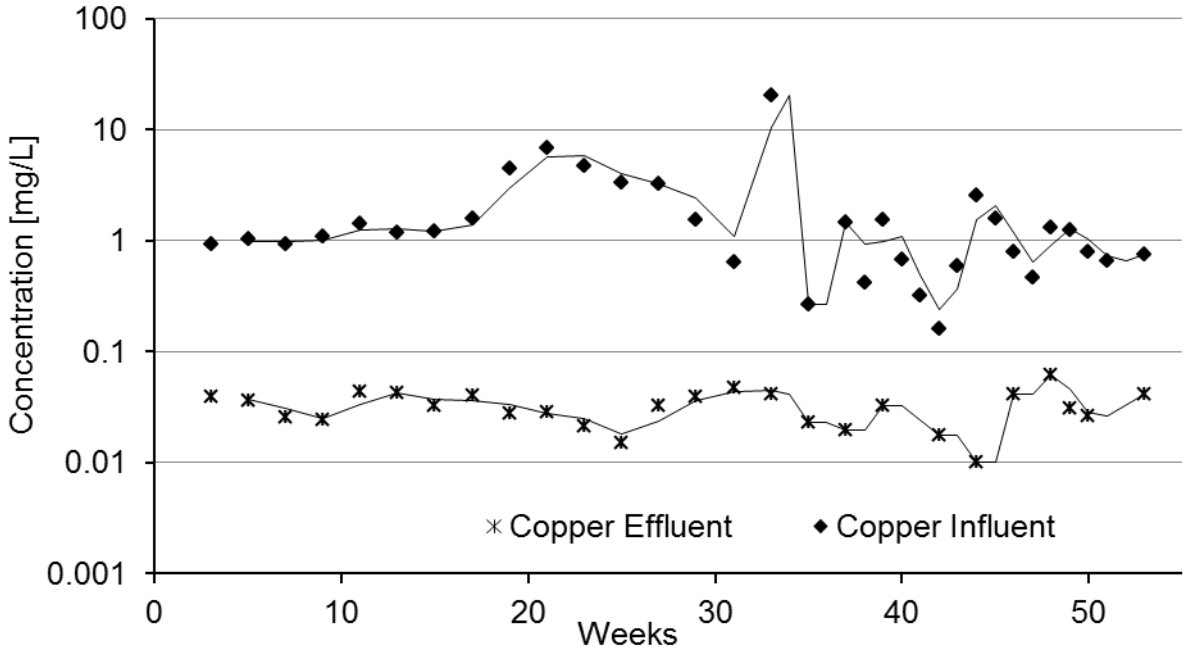


Figure 7.2 Concentration of Cu in the influent and effluent of the UF-PP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 26, effluent)

The concentration of Cu in the effluent was invariably in the range of 0.01 to 0.1 mg/L. The effluent data for Cu (Fig. 7.2) allow distinction of several periods related, in some cases, to the operation mode of the system:

- continuous gradual decrease between week 17 and week 25; influent concentration of Cu (and also Ni and Zn) was increased from 1 mg/L to 5 mg/L in week 18;
- increase between weeks 27−33 up to the level characteristic for weeks 10−15 of operation;
- unstable decrease in weeks 33−44; influent flow rate was decreased from 3.25 mL/min to 2.00 mL/min in week 33;
- noticeable increase at the end of the experiment (weeks 48−53) when cyanides were added to the feed.

It can be also observed that the influent concentration of Cu was fluctuating at a level of ca. 1 mg/L, which was lower than the intended value of 5 mg/L. The cause for these discrepancies could have been precipitation of copper hydroxide ($Cu(OH)_2$) in the feed container. The soulubility of this compound is the lowest at pH about 8.5, which was measured in the influent (Lewis, 2010).

In the same manner as for Cu Fig 7.3 presents influent and effluent concentrations of Ni in the UF-PP CWs during the experiment. The trendlines represent two-period moving averages.

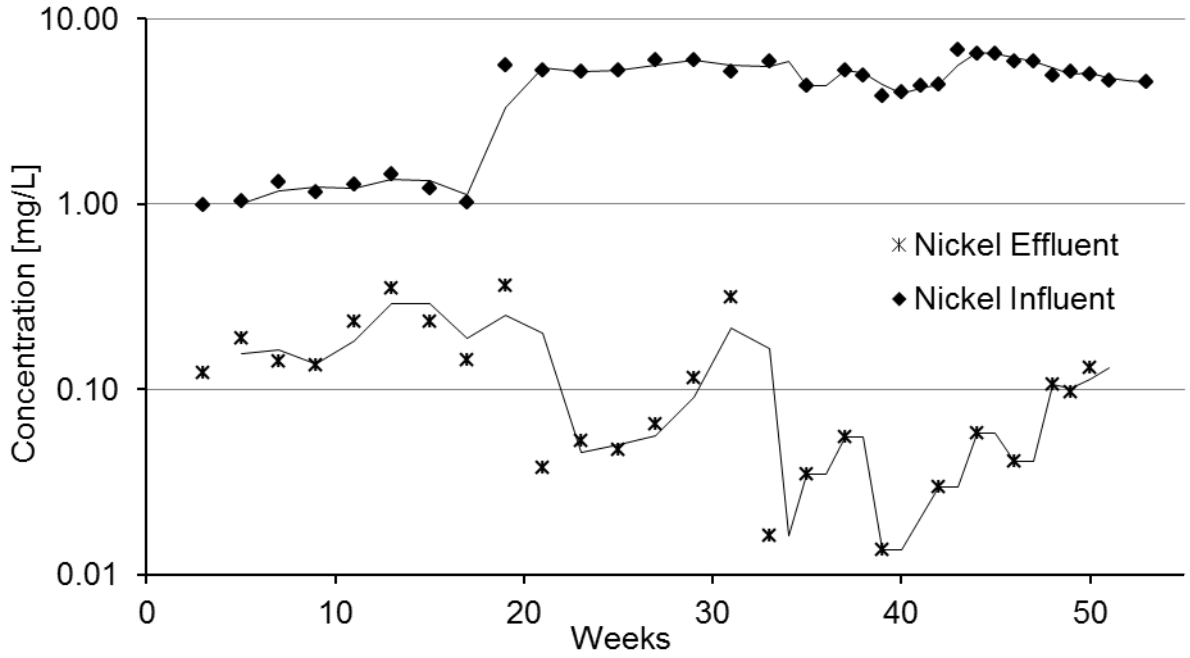


Figure 7.3 Concentration of Ni in the influent and effluent of the UF-PP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 25, effluent)

It can be noticed that unlike for Cu (Fig. 7.2), the influent concentrations are closer to the intended values (1 mg/L in weeks 1−17, and 5 mg/L in weeks 18−53), and the effluent concentration are more scattered often exceeding 0.1 mg/L, which was not a case for Cu. On analysing the distribution of the data points for Ni on the time scale several periods can be observed based on the effluent results:

- effluent concentrations before the increase of the influent Ni concentration from 1 to 5 mg/L in week 18 can be divided into two periods (before and after week 10). The Ni concentrations were lower in the period occuring directly after the start-up of the system than they increased;
- sudden decrease of the eflluent Ni concentration occurred in week 21 and than it was increasing gradually until week 31 to reach a value similar to this from week 19; influent concentration of Ni (and also Cu and Zn) was increased from 1 mg/L to 5 mg/L in week 18;
- another sudden decrease in the effluent concentration occurred in week 33 when the inflow rate was set to 2.00 mL/min instead of the previous value of 3.25 mL/min, thus extending the HRT in the system;
- after week 33 most of the data points for the effluent concentration were below 0.1 mg/L, however, it can be seen that at the end of the experiment the effluent concentration

increased above 0.1 mg/L, which can be attributed to the addition of cyanides to the influent in week 48 (and on) of the experiment.

Lead was the only metal that was maintained at the same level (1 mg/L) throughout the experiment. The time-dependent influent and effluent concentrations of Pb are shown in Fig. 7.4 for the entire duration of the experiment. The trendlines represent two-period moving averages.

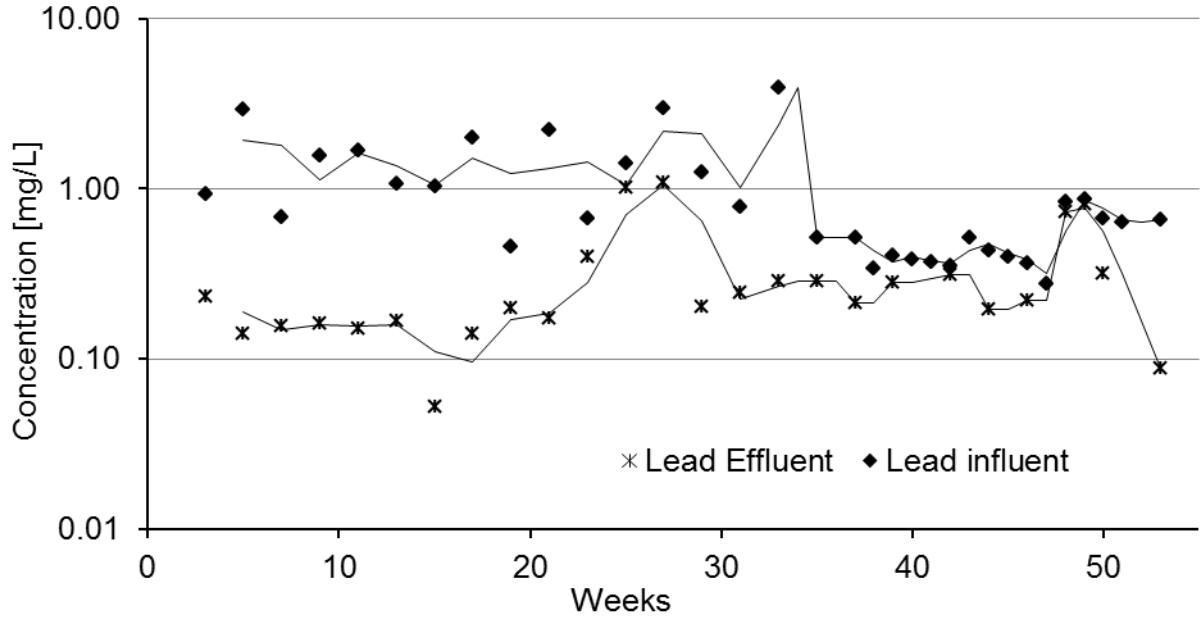


Figure 7.4 Concentration of Pb in the influent and effluent of the UF-PP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 26, effluent)

It can be seen in Fig. 7.4 that the effluent concentration had a slightly increasing tendency when the whole time scale is considered. It may be caused by the internal factors (saturation of adsorption sites) or external factors such as the adjustments to concentration of metals in the feed (week 17) or the decrease of flow rate (week 33). In general, it can be observed that the treatment efficiency is decreasing in the course of the experiment. It may be assumed that the response of the system is independent of the influent concentration, which can be observed, for example, in weeks 35−46 when the influent concentration of Pb is below the intended value and the effluent concentration remains at a similar level as for higher concentration of Pb in the influent. In addition to the general increasing tendency for the effluent Pb concentration it can be seen that there are two peaks when the Pb concentration increased significantly above the average tendency. These occurred between weeks 23−29, which can be attributed to a time-shifted response to adjustment of the feed concentration of metals (week 17), and then in weeks 48−49, which may be related to the addition of cyanides in weeks 48−53.

Figure 7.5 presents the influent and effluent concentrations of Zn in the UF-PP CWs investigated in the study. The trendlines represent two-period moving averages.

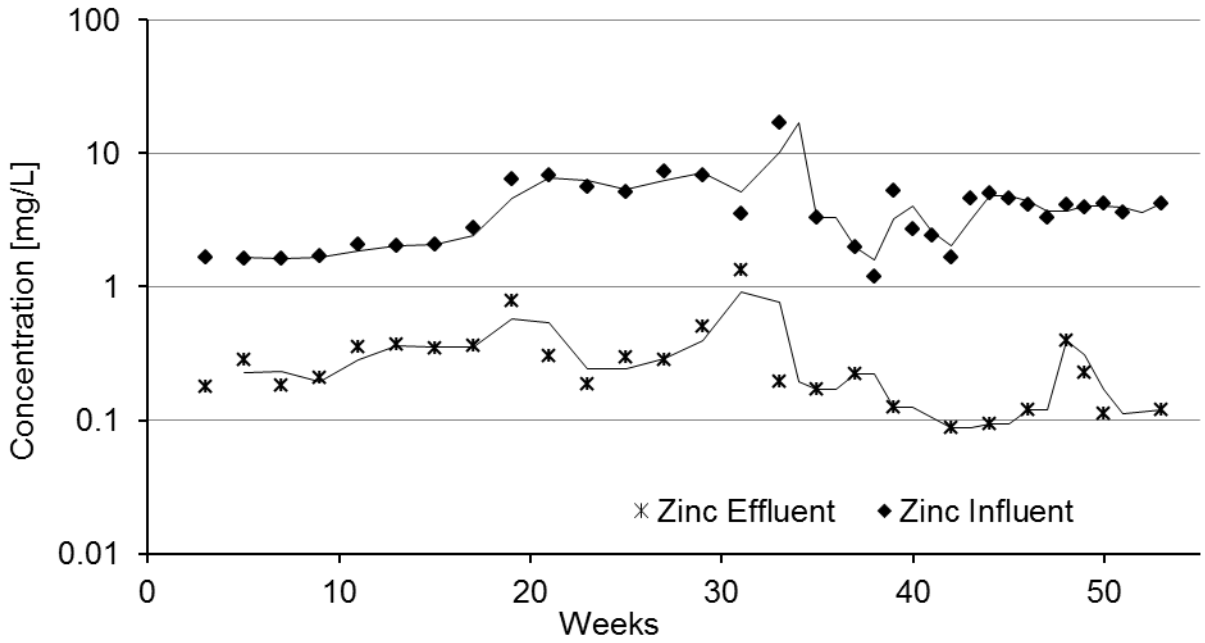


Figure 7.5 Concentration of Zn in the influent and effluent of the UF-PP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 26, effluent)

It can be seen in Fig. 7.5 that there are several treatment periods into which the overall tratment can be divided:

- in the period before week 17 the effluent concentration of Zn was on a relatively constant level;
- a certain increase of the Zn concentration in effluent was observed in week 19 after the concentration of Zn in the feed was increased from 1 to 5 mg/L;
- after week 23 the Zn concentration was increasing gradually in a manner similar to the observed for Ni in the same period (Fig. 7.3);
- from week 33 (when the inflow rate was decreased) a gradual decrease of the influent Zn concentration was observed with a pseudo steady-state reached in week 42−46 and 50−53 and disrupted in week 48 proabably due to the addition of cyanides to the feed.

Most of the data points for the Zn concentration in the effluent (23 of 26) are within the range of 0.1 to 1 mg/L. In general, within the studied period, it can be observed that the effluent concetrations follow sligtly decreasing tendency towards the end of the experiment. Thus, it can be assumed that the treatment efficiency was incraesing towards the end of the experiment, since the influent concentration was at a constant level.

7.2.3 Treatment efficiency of the UF-GP columns

The UF-GP CWs studied during the experiment had only mineral bed medium and were operated similar to the UF-PP columns with the exception that lactates were being added to the feed as an external carbon source (week 44 and on). The results including the influent and effluent concentrations of metals presented as a function of time allow observation of the system behaviour during the whole experiment (Fig. 7.6−7.9).

Figure 7.6 presents the influent and effluent Cu concentration for the UF-GP CWs during the whole duration of the experiment. The trendlines represent two-period moving averages.

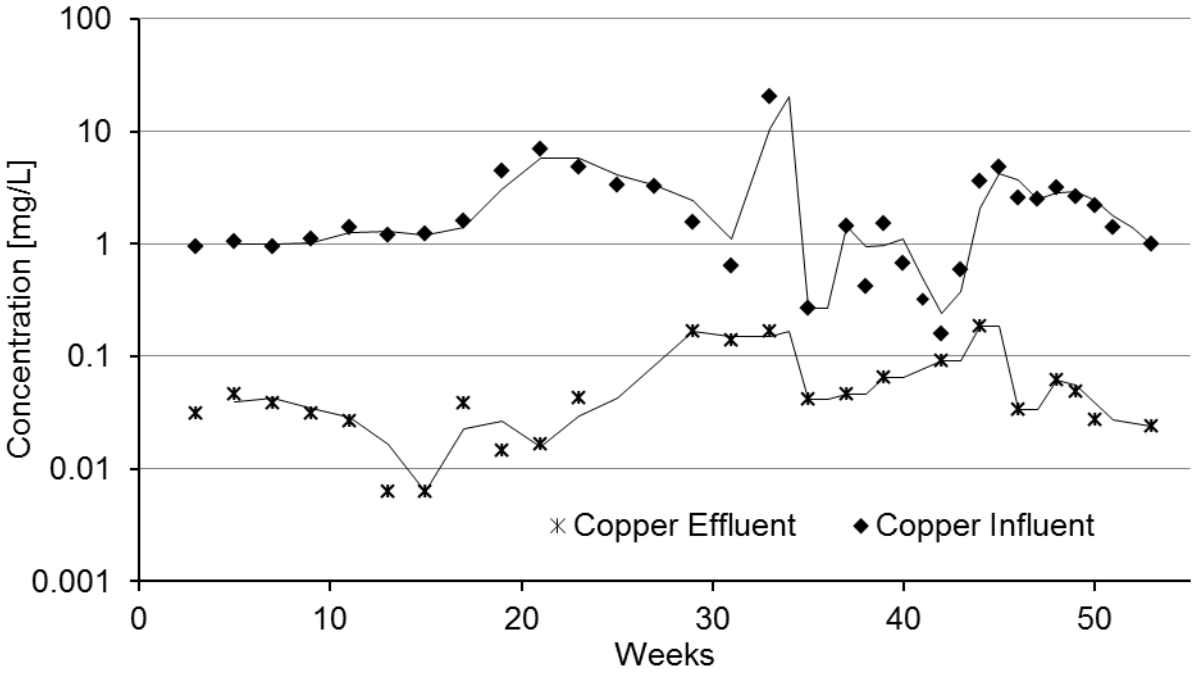


Figure 7.6 Concentration of Cu in the influent and effluent of the UF-GP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 24, effluent)

It is diffucult to assume a general tendency for the effluent concentration of Cu. It can be observed that most of the data points (18 of 24) are within the range of 0.01−0.1 mg/L. Referring the effluent concetration of Cu to the operational conditions it can be stated that:

- after relatively stable operation of the system before week 11, then fluctuations were observed followed by gradual increase of the Cu concentration probably due to the increase of the Cu concentration in the feed from 1 to 5 mg/L;
- in weeks 29-33 the system reached a pseudo steady-state, however it was a period of low treatment efficiency;

- after week 33 (when the inflow rate was decreased from 3.25 to 2.00 mL/min) appeared a sudden decrease of the Cu concentration, after which the concentration of Cu was increasing up to the level observed in weeks 29−33;
- there are two interesting observations for a period between weeks 46 and 53: firstly, the Cu concentration decreased markedly, which can be attributed to the addition of lactates in week 44), and secondly a small peak can be observed between weeks 48 and 50, which is probabaly because cyanides were started to be added in week 48. Such a cyanide-induced peak was also observed in the effluent from the UF-PP CWs (Fig. 7.2).

The influent and effluent data points for Ni in the UF-GP system along the time scale are presented in Fig. 7.7.

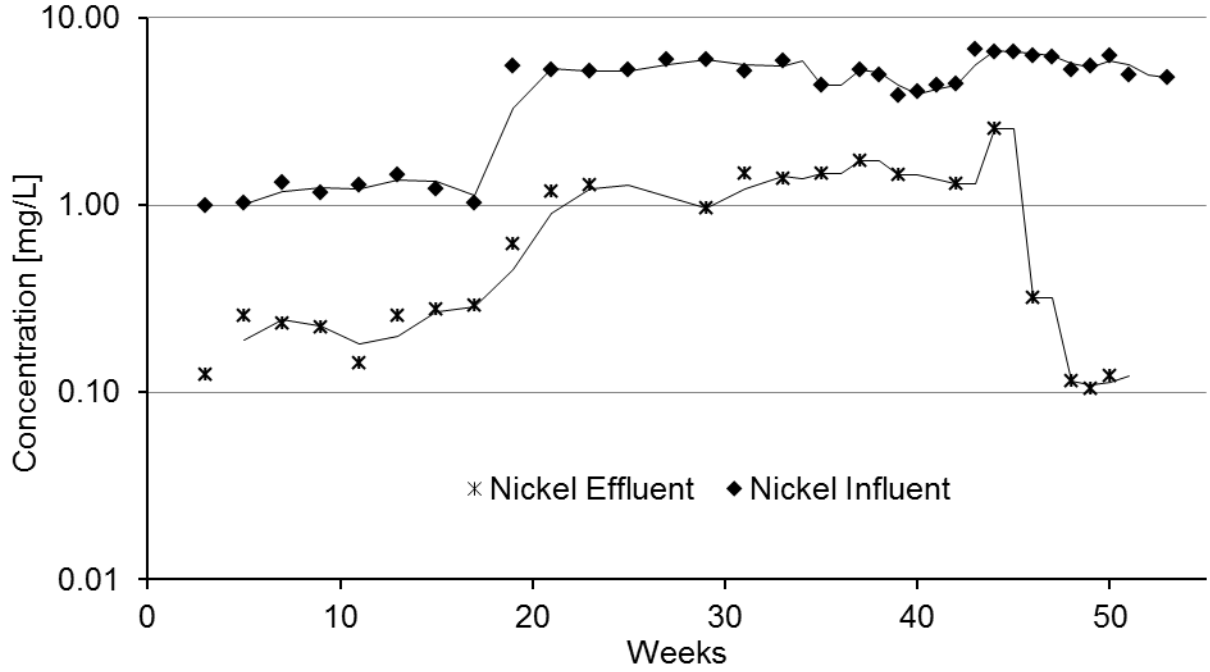


Figure 7.7 Concentration of Ni in the influent and effluent of the UF-GP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 23, effluent)

The general tendency of the Ni removal in the UF-GP CWs is that the effluent concentration appears to be proportional to the influent concentration. This observation is, however, not valid at the end of the experiment (week 44 and on) when lactates were started to be added to the influent, which allowed achieving the highest removal efficiency observed during the experiment. The behaviour of the UF-GP CWs before week 44 is in contrast to the observation made for the UF-PP CWs (Fig. 7.3), where the removal efficiency increased when the influent concentration of Ni was increased, and the resulting effluent concentration was below 0.1 mg/L. As it can be seen in Fig. 7.7 the effluent concentration of Ni in the UF-GP CWs increased by the

same proportion as the increased influent concentration and then it was slightly increasing until lactates were added to the influent.

The concentration of Pb in the influent and effluent of the UF-GP CWs during the whole experiment is shown in Fig. 7.8.

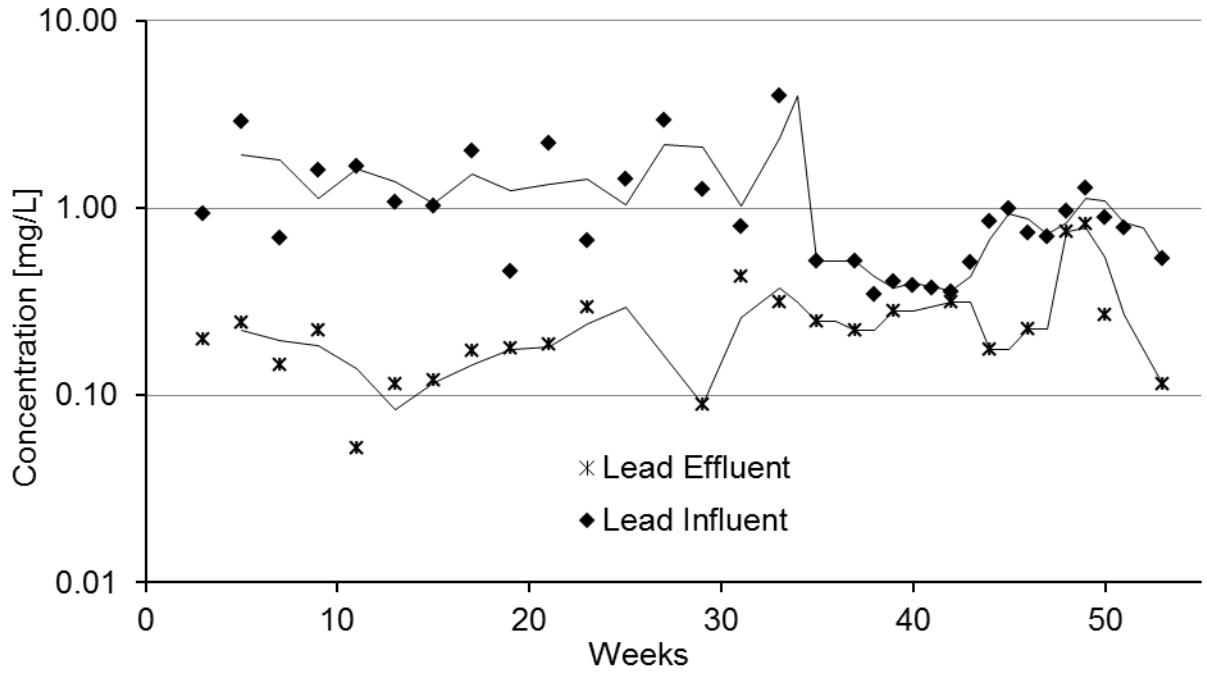


Figure 7.8 Concentration of Pb in the influent and effluent of the UF-GP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 24, effluent)

Most of the data points for the effluent Pb (22 of 24) concentration are within the range of 0.1−1.0 mg/L. It can be observed that the effluent concentration of Pb was increasing with the time course until week 44, when lactates were started to be added to the influent. The addition of lactates caused a decrease of the Pb concentration in the effluent, which was observed until the end of the experiment, with a peak in weeks 48−50, probably due to the addition of cyanides. It should be mentioned that the data points for the effluents of the UF-GP and UF-PP CWs (Fig. 7.4) lie surprisingly close to each other in weeks 30−53. The decreasing effluent concentration of Pb in the UF-GP CW after week 44 can be attributed to the presence of lactates in the influent, but the same phenomenon observed in the UF-PP cannot be similarly explained. It is merely a hypothesis but perhaps the decrease of the Pb concentration in the effluent of both UF-PP and UF-GP could have been caused by the presence of cyanides in the influent, but they were started to be added only in week 48.

The influent and effluent data regarding Zn concentration are given is Fig. 7.9 for the whole duration of the experiment.

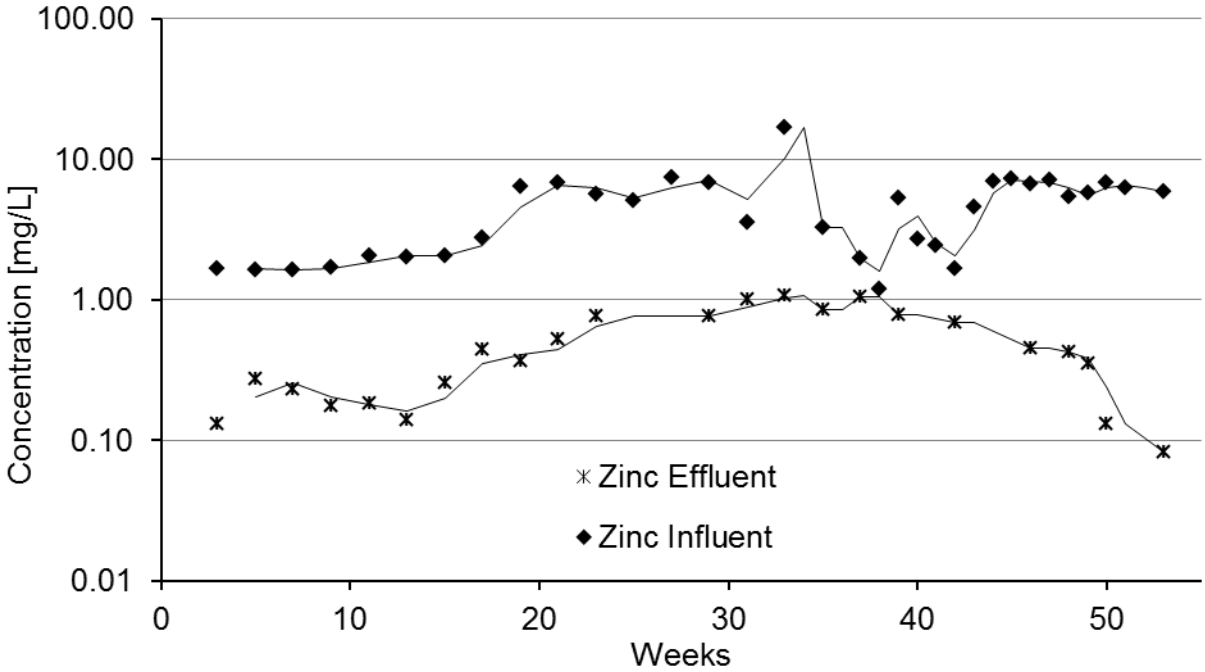


Figure 7.9 Concentration of Zn in the influent and effluent of the UF-GP CWs as a function of time for the whole duration of the experiment (weeks 1−53), (n = 33, influent; n = 23, effluent)

It can be seen that the effluent Zn concentration had an increasing tendency until week 37, after which it started to decrease with a gentle slope until week 49. It can be also noticed that the increase of the Zn concentration (and also Ni and Cu) in the influent from 1 to 5 mg/L (week 18), and then the decrease of the inflow rate (from 3.25 to 2.00 mg/L in week 33) did not have a pronounced and sudden effect on the effluent quality in terms of Zn concentration. It is, indeed, noticeable that the increase of Zn concentration in the effluent occurred after week 18 but this tendency started before and it is difficult to explain if it can be attributed to the effect of the adjusted influent or, for example, saturation of the adsorption sites in the bed medium. When the effect of the smaller inflow rate is considered it can be seen that the concentration of Zn in the effluent started decreasing with a certain time shift. The same observation is valid for the effect of lactates, which were started to be added in week 44, but a significant decrease of the Zn concentration in the effluent was observed only in week 50, and, noteworthy, it cannot be excluded that it was caused by the addition of cyanides (week 49). It should be mentioned, however, that there were at least two external factors occurring in parallel, so that it is practically impossible to distinguish their effect by analysing the effluent concentration data. This is a case, for example, for the period between weeks 37 and 49, when the decrease of the effluent

concentration of Zn could have been affected by the reduced inflow rate and then the addition of lactates (week 44). This remark is also valid for the other elements studied and could have occured in the other CWs used during the experiment.

7.3 Treatment efficiency of the constructed wetlands for cyanides

Cyanides are an inherent compound of electroplating wastewater, however, their presence can be detected only in partly treated wastewater, which was observed based on the real-world data from the electroplating plant of the PEM. In the experiment in system B cyanides were added to the high-strength wastewater starting from week 48. The influent and effluent cyanide concentration and the removal efficiencies are presented in Tab. 7.3. The removal efficiencies given in Tab. 7.3 were calculated based on median influent and effluent concentrations. The influent concentration of cyanides were different for columns with mineral and organic media, as these two types of columns were fed with wastewater with lactate and without lactate, respectively, from separate tanks

Table 7.3 The influent and effluent concentrations (median±MAD) of readily liberated cyanides an corresponding removal efficiencies in all the types of columns (weeks 48−53; n = 5−8)

		Column type					
		UF-PP	**UF-PU**	**UF-GP**	**UF-GU**	**FaD-PP**	**FaD-GP**
Influent	**Median**	1.043[1]	1.043[1]	1.280[2]	1.280[2]	1.043[1]	1.280[2]
mg/L	**MAD**	0.534	0.534	0.851	0.851	0.534	0.851
Effluent	**Median**	0.056	0.085	0.085	0.080	0.198	0.180
mg/L	**MAD**	0.043	0.065	0.044	0.028	0.106	0.154
Removal							
%		94.7%	91.9%	93.4%	93.8%	81.0%	86.0%

[1]for the feed without lactates, [2]for the feed with lactates

The results presented in Tab. 7.3 indicate that the removal of cyanides was higher than 91% in the UF columns and it was 81% for the FaD-PP CW and 86% for the FaD-GP CWs. Importantly, only the UF-CWs allowed to meet the Polish legal standards that set maximum allowable concentration for free cyanides in wastewater discharged into the environment at 0.1 mg/L (PL MoE, 2006). It appears that continuously anoxic conditions in the UF CWs were more advantageous than intermittently changing oxic and anoxic conditions in the FaD CWs. All the effluent concentrations were statistically different from the corresponding influent concentrations.

7.4 Constructed wetlands as an emergency system

In case of a failure of an alkaline buffer tank and capacity shortage of the whole system a CW could serve as a receiver of excess wastewater and what is more a buffer before highly toxic wastewater is discharged into the environment. This situation was simulated as a one-off event during the experiment in system B. Only the FaD CWs were used to study the treatment efficiency for the raw alkaline wastewater to diminish the risk associated with using such aggressive medium. The composition of the synthetic wastewater (Fig. 7.4) was based on the quality of the real raw wastewater fed into the alkaline buffer tank (Fig. 3.1) according to the data obtained from the PEM plant (SOFIPEM, 2009). Table 7.4 shows the influent and effluent data and the resulting effect of the treatment of the raw alkaline wastewater. The removal efficiencies were calculated based on the data for the filtered influent and effluent samples. The concentrations of metals and cyanides based on the amounts of the salts added to the feed are also given in Tab. 7.4. The duration of a holding phase was 7 d.

Table 7.4 Influent and effluent data and the removal efficiencies for the FaD CWs during the discharge of the raw alkaline wastewater, system B

		Pb	Zn	Cu	Ni	CN	pH
FaD-PP	**Effluent conc.[2], mg/L**	0.86	0.93	2.32	4.30	3.30	7.6
	Removal, %	-104.8%	-2225%	-55.7%	65.8%	87.3%	
FaD-GP	**Effluent conc.[2], mg/L**	0.20	0.43	8.02	5.12	3.50	10.8
	Removal, %	52.4%	-975%	-438%	59.2%	86.5%	
Influent (filtered)[2], mg/L		0.42	0.04	1.49	12.56	26.00	13.0
Influent (calculated)[1], mg/L		2.00	3.50	39.00	32.00	37.00	

[1]total cyanides based on the mass of the salts added to the feed, [2] measured as readily liberated cyanides

The results presented in Tab. 7.4. indicate that predominant fraction of metals were their hydroxide precipitates, which were present in the tank where the feed was prepared. The fraction of metals that was analysed was only the filtered one. The removal efficiency for the metals present in the feed is positive only for Ni in both types of columns and Pb in the FaD-GP columns. Copper and Zn were released from both types of the columns and Pb was released from the FaD-PP CW. The actual removal of total (dissolved and particulate) metals could be higher because, as it was observed, the metal hydroxides present in the feed were filtered out on the bed surface of all the FaD columns forming a filter cake. The release of metals (in forms that pass through a filter) from the columns can be attributed to analytical or physical-chemical issues. Considering the analytical issues it should be mentioned that due to the fact that total metal concentrations were unknown (only the filtered samples were analysed) it is impossible to

determine whether the actual removal was positive or negative for the total or particulate metal forms. The particulate fraction of metals can be calculated only for the influent by deducting the concentration in filtered samples from the concentration corresponding to the amount of salts added to the feed. The information on the amount of particulate forms of metals for the influent cannot be compared with the effluent data, as the amount of undissolved metal forms in the effluent was neither determined nor can be calculated. Based on the results given in Tab. 7.4 it can be seen that the concentration of soluble forms of metals increased during the passage through the system. It may stem from the fact that the solubility of metal hydroxides and sulphides increases at alkaline conditions (Lewis, 2010). Regarding the difference between the calculated (by dissolving KCN) and the measured concentration of readily liberated cyanides it can be seen that ca. 11 mg/L of cyanides were present as non-readily liberated cyanide species, probably complexed with metals. The removal efficiency for cyanides was comparable in both CWs, 87.3% and 86.5%, for the FaD-PP and FaD-GP CWs, respectively. Interestingly, the cyanide removal efficacy in the FaD-GP columns was similar to the median reduction (86.0%) in the period when these columns were fed with the influent containing markedly lower concentration of cyanide, this is, 26.00 mg/L and 1.026 mg/L, for raw alkaline wastewater and for high-strength influent, respectively (Tab. 7.3 and 7.4). The pH value was very high as for the wastewater to be discharged into CWs and is rarely reported in the literature (Mayes et al., 2009). The pH of the influent was reduced from 13.0 to 10.8 and 7.6 after one-week holding time in the FaD-GP and FaD-PP CW, respectively. It indicates that CWs have the potential of buffering highly alkaline wastewater, which was also suggested by Mayes et al. (2009). In this one-off event the columns filled with peat proved to be more efficient in neutralizing the wastewater comparing to gravel. It should be mentioned, however, that the results obtained for a single batch cannot be conclusive considering long-term treatment.

It is not only the efficiency of the CW during the emergency event that should be considered. It should be borne in mind that the aggressive properties of the raw wastewater may compromise further treatment in the system. In this experiment the high-strength influent was again fed to the FaD columns to observe whether the simulated raw wastewater had an adverse effect on the performance of these columns. The mean treatment efficiency after this simulated emergency event is presented in Tab. 7.5

Table 7.5 Comparison between removal efficiencies of metals and cyanide before (weeks 44−49) and after (weeks 52−53) the simulated emergency event, system B

Element/Compound	Removal, %	
	Column FaD-PP	Column FaD-GP

	Before	After	Before	After
Cu	58.0	-19.3	93.0	-14.5
Ni	93.7	86.3	83.9	91.1
Pb	54.7	70.0	71.1	76.4
Zn	73.7	84.6	92.6	93.8
Cyanide[1]	86.4	89.1	97.4	86.4

[1]readily liberated cyanide

It can be seen in Tab. 7.5 that the FaD columns were not adversely affected during the emergency event in terms of the removal efficiency of cyanide and metals. The only exception is Cu that was released from both FaD columns treating the high-strength influent after the emergency event. It was not studied if this effect was reversible.

7.5 Effect of operational conditions on the treatment efficiency (system B)

The design of the experimental system was intended to allow for comparison of the effect of various operational conditions and configuration of the columns. The overall averaged data for the whole experiment or the data presented on the time scale are not suitable for analysing the results in this regard. In this section the effect of various factors is assessed based on the data paired according to the effect of interest. This is, each pair comprises two data sets, one set where the factor with a potential effect on the treatment efficiency occurred and the other set, in which this factor was absent. The analysed data were divided into relevant periods to enable meaningful conclusions drawn from the comparison. The significance of the difference between two relevant data sets was assessed statistically.

7.5.1 Effect of wastewater strength

The experimental system was fed with either low- or high-strength wastewater, which was intended to simulate the treated electroplating effluent and pretreated electroplating effluent, respectively. The low-strength influent was fed at the beginning of the experiment (weeks 1−17) and the high-strength was fed in the remaining period (weeks 18−53). The results from these periods were compared to assess whether the strength of the influent affected the treatment efficiency. It should be noted that these two influents were not fed into the columns in parallel, so the apparent difference between the treatment efficiencies in these periods cannot be solely attributed to the effect of the wastewater strength but could have also been caused by the system's maturation. The comparison of the removal efficiency of the UF columns between the periods when the system was fed with low- and high-strength influent is presented in Tab. 7.6. The data shown for the high-strength feed was for weeks 18-32, before inflow rate was decreased.

Table 7.6 Comparison between the treatment efficiencies (Median ± MAD, %) in the UF-PP and UF-GP columns fed with low- (Low) and high-strength influent (High), (weeks: 1−17 for low-strength influent, n = 8; 18−32 for high-strength influent, n = 8), system B

Element	UF-PP		UF-GP	
	Low	**High**	**Low**	**High**
Cu	**97.1±0.5**	**99.5±0.2**	97.4±1.1	99.2±0.6
Ni	**83.9±3.3**	**99.0±0.6**	81.5±5.1	76.0±2.9
Pb	**90.4±4.7**	**66.0±21.6**	88.9±2.9	58.4±14.3
Zn	**85.2±2.5**	**94.9±2.1**	88.5±3.3	92.3±5.9

Statistically significant differences are shown in bold

Based on the results presented in Tab. 7.6 it can be seen that all the differences between the removal efficiencies in the given periods for the UF-PP columns were statistically significant, in contrast to the to the UF-GP columns where none of the differences were of statistical significance. In the high-strength-influent period the operation of the UF-GP was unstable for two weeks because of tubing clogging and puncture, which could cause significant vartiations in the data set for this period. It was, observed however, that the type of influent affected the operation of the UF columns in a similar manner. The removal of Cu was ca. 2% higher, in both UF columns, when the high-strength influent was used. Also, the removal of Ni was more efficient, by ca. 16%, when the high-strength influent was fed to the UF-PP columns. It was the only exception to the similarity between the UF columns, when the Ni removal was lower by 5.5% during the period when the high-strength influent was fed to the UF-GP columns. It was probably owing to saturation of the gravel, which has lower sorption capacity than peat. The same observation as for Cu and Ni (only in the UF-PP columns) was observed for Zn, which was removed to a higher extent in both types of the planted UF columns when these columns were fed with the high-strength influent. The efficiency of the Zn removal increased by ca. 10% and 4% for the UF-PP and UF-GP CWs, respectively. In contrast to the above findings (with the exception of the Ni removal in the UF-GP CWs), the Pb removal efficiency was markedly lower, by ca. 30% and 24%, in the UF-PP and UF-GP CWs, respectively, when the high-strength wastewater was fed into these CWs. However, in this period influent concentrations of only Cu, Ni and Zn were increased from 1 to 5 mg/L, but Pb concentration remained unadjusted at 1 mg/L. This may suggest that the Pb removal was hindered by the increased concentration of the other metals.

The comparison of the removal efficiency of the FaD columns between the periods when the system was fed with low- and high-strength influent is presented in Tab. 7.7.

Table 7.7 Comparison between the treatment efficiencies (Median ± MAD, %) in the FaD-PP and FaD-GP columns fed with the low- and high-strength influent, (weeks: 1−17 for the low-strength influent, n = 7; 18−32 for the high-strength influent, n = 7), system B

Element	FaD-PP		FaD-GP	
	Low	**High**	**Low**	**High**
Cu	81.5±4.5	93.4±5.2	97.9±1.0	97.0±2.3
Ni	89.2±0.8	93.4±2.5	94.5±0.2	96.6±0.9
Pb	90.1±4.5	76.7±9.0	83.0±4.1	75.1±14.7
Zn	27.2±41.2	57.0±33.8	81.4±3.4	88.5±6.0

No statistically significant differences observed

In the case of the metal removal in the FaD columns similar regularities were observed as for the planted UF columns. No statistically significant differences were observed between the data subsets for the FaD-PP nor for the FaD-GP columns. The removal efficiency of Cu increased by 12% in the high-strength period in the FaD-PP column, and decreased by less than 1% in the FaD-GP columns. Ni was removed with an increased efficiency during the period when the high-strength influent was fed to the system and the difference comparing with the efficiency corresponding to the low-strength influent was higher by about 4% for the UF-PP CWs and by about 2% for the UF-GP CWs. Similarly as in the planted UF columns, the removal of Zn in both types of the FaD columns was enhanced when they were fed with the high-strength influent, by about 30% for the FaD-PP CW, and by about 7% for the FaD-GP CWs. Interestingly, similarly to the observation made for the Pb removal in the planted UF CWs, in the FaD CWs the Pb removal was reduced in the period when the high-strength influent was used. This corroborates the hypothesis that the increased concentration of Cu, Ni and Zn (except Pb) attenuated the removal of Pb. However, it cannot be stated which is the mechanism of Pb removal that is hindered most, for example, whether it is competition for the sorption sites or the biogenic sulphides.

7.5.2 Effect of vegetation

Plants are an inherent feature of CWs (Brix, 1997). The significance of their effect on the removal of metals depends on many factors, which was discussed in Section 5.1.3. The effect of plants on the removal of metals in system B is presented in Tab. 7.8 where the data for the planted and unplanted UF columns are compared.

Table 7.8 Comparison between the treatment efficiencies (Median ± MAD, %) in the planted and unplanted UF columns, (weeks 3−42; n = 18−21), system B

Element	UF-Peat		UF-Gravel	
	Planted (UF-PP)	**Unplanted (UF-PU)**	**Planted (UF-GP)**	**Unplanted (UF-GU)**
Cu	**97.5±1.5**	**96.5±2.4**	**97.0±2.2**	**97.5±1.7**
Ni	**96.0±3.5**	**88.8±4.7**	**76.0±5.7**	**84.3±2.6**
Pb	76.1±6.7	80.1±11.0	78.8±15.4	80.8±11.5
Zn	**89.1±5.7**	**87.5±4.3**	**86.4±5.9**	**92.7±3.3**

Statistically significant differences are shown in bold

The differences between the data sets for the planted and unplanted counterparts columns were statistically significant for all the metals, except Pb. The differences between the removal of Cu in the planted and unplanted UF columns filled with either gravel or peat are less than 1%. The results for Ni are somewhat contradictory, as the presence of plants enhanced the treatment efficiency in the UF-PP columns by ca. 7.2%, but it attenuated the removal of Ni in the UF-GP CWs by about 8%. The same observation is valid for the removal of Zn, which was higher (by only 1.6%) in the UF-PP CWs than in the unplanted counterpart (UF-PU CWs), but was lower, by about 6%, in the UF-GP than in the unplanted counterpart (UF-GU CWs). The removal of Pb was higher in the unplanted UF columns, both in these filled with gravel or peat, and differed by 4% and 2%, respectively.

It should be mentioned that the plants in system B in the investigated period were in the initial period of their growth. The maximum height of a stem in the end of week 42 was about 50 cm. The plants were planted as rhizomes, therefore their growth was not as rapid as in case of the seedlings used in system A. Also the indoor conditions in which system B was operated were not as advantageous for the growth of plants as in the case of system A, which was operated in the outdoor conditions since the plants were planted in May. As it was mentioned in Section 6.2.1 the amount of data for the UF columns of system A is scarce because of the failure of the pumping system. It is interesting, however, to present a single data set (one-off sampling) for the counterpart columns of system A after 9 weeks of the system's operation when the plants were about 1.0 m high (Fig. 7.10).

Figure 7.10 Experimental system A after 9 weeks of operation (August 2011)

The results of the treatment efficiency in system A in week 9 of the operation are given in Tab. 7.9. for Cu, Ni and Zn. The Pb concentration was not measured.

Table 7.9 The removal efficiency (%) of the UF columns of system A in week 9 of the experiment and the influent concentration of elements (mg/L)

Element	Influent	UF-Compost		UF-Sand	
		Planted (UF-CP)	Unplanted (UF-CU)	Planted (UF-SP)	Unplanted (UF-SU)
Cu	0.812	97.1	98.7	97.7	94.3
Ni	0.173	90.8	93.3	94.9	87.2
Zn	0.123	79.4	76.2	82.5	85.1

It can be seen that in the initial period of the system's operation the removal of Cu was higher in the UF-SP columns than in their unplanted counterparts, in the columns with organic media the removal was higher in the unplanted columns (UF-CU) than in the planted ones (UF-CP). The same regularities are valid for the removal of Ni. In contrast to the behaviour of Cu and Ni, Zn was removed with higher efficiency in the planted UF columns with compost (UF-CP) than in

the unplanted counterpart (UF-CU), but its removal efficiency was lower in the planted columns with sand (UF-SP) than in the unplanted ones (UF-SU). The comparison of the results for system B (Tab. 7.8) with the results for system A (Tab. 7.9) indicates that the only similarity is the behaviour of the systems towards Zn, but it differed for Cu and Ni. The planted UF columns with organic media and the unplanted UF columns with mineral media were more efficient in the removal of Cu and Ni in system B than their counterparts in terms of the presence of plants. Conversely, the unplanted UF columns with organic media and the planted columns with mineral media were the most efficient in system A. It is difficult to satisfactorily explain the cause of the difference between systems A and B. It cannot be excluded that other components of the system than plants can play a major role causing the difference, for example, the properties of the bed media and quality of wastewater. It is noteworthy that the influent fed to system A in week contained lowe concentrations of Cu, Ni and Zn (Tab. 7.8) than the influent of system B, which contained about 1-5 mg/L of Cu, Ni and Zn (depending on the period). When only the role of the plants is considered it should be mentioned that it could have been different for system A and B. In system A the well-developed biomass of the common reed could have markedly modified the oxidation-reduction conditions in the bed media, so that oxic process could have been predominant in the upper zones of the columns. Also the high stem density in the water layer could have provided additional substrate for the biofilm that participated in the treatment processes. In system B, by comparison, the number of stems was less than 10, thus probably, the below-ground biomass was insufficient as a substrate for biofilm and as a means of oxygen transfer into the bed media. Thus, neither biotic nor abiotic processes occurring in the planted columns of system B were not affected significantly by the plants. The differences observed between the removal efficiencies in the planted and unplanted columns of system B were probably due to the fact that the plants modified the hydraulic permeability of the upper part of the columns, thus affecting the removal.

7.5.3 Effect of operation mode

Two operation modes were selected for the experiment: continuous upflow (UF) mode and fill-and-drain mode (FaD) (Section 6.1). The UF mode was intended to promote anoxic conditions, which would enable the process of sulphate reduction and subsequent precipitation of metals with sulphides. The fill-and-drain mode promotes alternating anoxic and oxic conditions, so that metals can be removed as sulphides but also as oxides and hydroxides. The comparison of the FaD and UF columns is not fully meaningful as the holding time and the HRT in these columns, respectively, were not equal. The HRT, in weeks 33 and on, for the UF columns was in the range

2.7-3.4 d, and the holding time in the FaD columns was 7 d. The comparison of the removal efficiency in the UF and FaD is given in Tab. 7.10. The statistical significance of the differences was not analysed as the data correspond to different retention time of wastewater in the columns.

Table 7.10 Comparison of metal removal efficiencies (Median ± MAD, %) in the UF and FaD columns, weeks 33−43 (gravel columns, n = 5−8), weeks 33−47 (peat columns, n = 7−11)

Element	Peat and plants		Gravel and plants	
	UF-PP	**FaD-PP**	**UF-GP**	**FaD-GP**
Cu	95.4±3.6	44.6±24.1	94.9±6.1	90.7±6.2
Ni	99.2±0.1	89.5±4.1	66.1±4.2	80.9±3.5
Pb	39.7±16.1	24.1±21.0	52.0±21.2	50.3±28.5
Zn	94.8±2.9	61.9±12.7	74.0±1.2	83.7±6.5

The results given in Tab. 7.10 suggest that the UF operation mode is much more efficient in the columns filled with peat, but only slightly more efficient for Cu and Zn in the columns filled with gravel, and less efficient for Ni and Zn. The differences between the UF and FaD filled with peat, were more pronounced than for the gravel columns. This is probably because of the clogging propensity of the FaD-PP column. The results for the gravel columns may suggest that the alternating conditions in the FaD-GP columns were not disadvantageous for the removal of metals. What is more the removal of Ni and Zn was probably enhanced by oxic processes, such as precipitation of oxides or hydroxides of metals.

7.5.4 Effect of hydraulic loading rate

The hydraulic loading rate is an operational parameter that can be used to control the efficiency of the treatment in CWs. The complexity of the processes occurring in CWs for the removal of metals and cyanides causes that it is difficult to assume any value of the hydraulic retention time in the beginning of the experiment as the optimum one. For this reason, two values of the hydraulic loading rate were studied, for which the results are given in Tab. 7.11. The influent quality was not modified in the period, for which the data are given.

Table 7.11 Comparison of metal removal efficiency (Median ± MAD, %) depending on the hydraulic loading in the UF columns, system B

Element	UF-PP[1]		UF-GP[2]	
	0.15 m/d	**0.09 m/d**	**0.15 m/d**	**0.09 m/d**
Cu	99.6±0.2	95.4±3.6	99.1±0.7	94.9±6.1
Ni	99.0±0.3	99.2±0.1	75.5±2.0	66.1±4.2
Pb	**69.1±38.3**	**39.7±16.1**	55.5±24.7	52.0±21.2
Zn	95.6±1.4	94.8±2.9	89.3±7.5	74.0±11.2

[1]weeks 21−32 (0.15 m/d; n = 7), weeks 33−47 (0.09 m/d, n = 7),
[2]weeks 21−32 (0.15 m/d; n = 7), weeks 33−43 (0.09 m/d, n = 5)
Statistically significant differences are shown in bold

The results given in Tab. 7.11 indicate that the treatment efficiency was lower for the lower values (0.09 m/d) of the hydraulic loading rate in both UF-PP and UF-GP columns. Only the removal of Ni in the UF-PP columns at both hydraulic loading rates was virtually equal. Most of the differences were not statistically significant with the exception of Pb in the UF-PP columns. The above findings suggest that the higher hydraulic loading rate is more advisable value rather than the lower one, however, two remarks should be made. Firstly, the decreased efficiency corresponding to the lower hydraulic loading rate can result from the chronology of the experiment. For example, the progressive saturation of the adsorption sites on the bed media could elucidate the decreased efficiency. Secondly, only two values of the hydraulic loading rate were investigated, therefore no reliable general conclusions can be drawn for a wider range of the values for this parameter.

7.5.5 Effect of the batch duration

The duration of the holding phase in the FaD columns has the implications for the treatment of the wastewater similar to the HRT in the UF columns. In the FaD it is, in contrast to the UF columns, the porosity of the bed media does not have to be determined to know the retention time, as it is equal to the batch duration. In the experiment the treatment of the wastewater was investigated at two different batch durations of one and two weeks. The two-week holding time was studied from week 1 to 32, and then the one-week batches were applied. The comparison of the treatment efficiencies for both periods is given in Tab. 7.12.

Table 7.12 Comparison of metal removal efficiency (Median ± MAD, %) depending on the batch duration of in the FaD columns, system B

Element	FaD-PP[1]		FaD-GP[2]	
	2-week batch	1-week batch	2-week batch	1-week batch
Cu	**95.3±3.7**	**44.6±24.1**	95.8±3.6	90.7±6.2
Ni	93.6±1.7	89.5±4.1	96.7±0.4	80.9±3.5
Pb	73.3±55.4	24.1±21.0	71.5±35.6	50.3±28.5
Zn	74.6±20.5	61.9±12.7	88.9±7.0	83.7±6.5

[1]weeks 21−32 (2-week batch, n = 7), weeks 33−47 (2-week batch, n = 11),
[2]weeks 21−32 (2-week batch, n = 7), weeks 33−43 (2-week batch, n = 8)
Statistically significant differences are shown in bold

Contrary to the behaviour of the UF columns (Tab. 7.11) the removal of metals was higher for the longer batch duration (2 weeks). The differences observed between the removal efficiency corresponding to the shorter and longer batches for the FaD-PP column were the most pronounced for Cu (the only statistically significant difference) and Pb, which were about 50% higher during the period of the two-week batches. The differences in the FaD-PP column were smaller for Ni and Zn, by about 4% and 13%, respectively, with higher efficiency during the period of the two-week batches. In the FaD-GP columns the differences between the removal efficiencies depending on the batch duration were generally smaller than in the FaD-PP column. The exception was for the removal efficiency of Ni which was higher in the FaD-GP columns. It is interesting that, as it was mentioned above, the treatment efficiency in the FaD columns increased with an increased retention time, and that, in contrast, it decreased with an increased retention time in the planted UF columns. Noteworthy, the holding time in the FaD columns was in a different range than in the UF columns, this is, 7−14 d and 1.6−3.4 d, respectively. It is difficult to draw any conclusions based on the comparison of the results for the UF and the FaD columns, as they were operating within different ranges of the HRT. It should also be mentioned that the chronology of adjusting the inflow rate for the UF columns, and the frequencies of fills and drains in the FaD columns, which determined the retention time, were different. The initial HRT in the UF columns was 1.6−2.1 d and in week 33 it was increased to 2.7−3.4 d, and the duration of the holding phase in the beginning of the experiment was 14 d, which was then shortened to 7 days in week 33. It should be taken into consideration because the progressive saturation of the adsorption sites on the bed media may cause that this effect would be construed as the effect of the adjusted hydraulic retention time. For both UF and FaD columns the decrease of the removal efficiency occurred in the latter period (after week 33) of the operation regardless of the HRT or the holding phase duration. It is, however, highly unlikely that the differences in the treatment efficiencies between these two periods would occur solely due to saturation of the bed media.

7.5.6 Effect of substrate type (internal carbon source)

The CWs have the ability to reduce metal concentrations in the wastewater passing through them. The removal of metals can occur under either oxic or anoxic conditions. In the experiment reported in this thesis the removal processes were investigated under anoxic and intermittently anoxic-oxic conditions. It was expected that the predominant anoxic removal process would be the precipitation of metals with biogenic sulphides. The generation of sulphides is mediated by SRB, which need organic carbon as an electron donor. The experimental system was designed to provide organic carbon as an internal source in the bed media, which was peat in system B. Not all of the columns of system B contained organic substrate so that the comparison of the effect of the internal carbon source would be possible. The data shown in Tab. 7.13 represent the treatment efficiency in weeks 3−42, this is, before the addition of external carbon source to the wastewater (discussed in Section 7.5.7). Table 7.13 presents the treatment efficiency comparison between the gravel- and peat-filled columns grouped according to the operation mode (UF or FaD).

Table 7.13 Comparison between the treatment efficiencies (Median ± MAD, %) in the columns filled with gravel (UF-GP and FaD-GP) or peat (UF-PP and FaD-PP), (weeks 3−42; n = 18−21), system B

Element	UF columns		FaD columns	
	UF-PP	**UF-GP**	**FaD-PP**	**FaD-GP**
Cu	97.5±1.5	97.0±2.2	**81.5±16.7**	**97.8±1.7**
Ni	**96.0±3.5**	**76.0±5.7**	90.0±3.4	94.4±3.1
Pb	76.1±16.7	78.8±15.4	75.8±17.3	78.8±13.2
Zn	89.1±5.7	86.4±5.9	**46.5±30.4**	**84.7±7.7**

Statistically significant differences are shown in bold

The effect of substrate type in the planted UF columns was statistically significant only for the removal of Ni, which was 20% lower in the columns with gravel (UF-GP). For the other metals that were determined the difference in the removal was less than 3% in the planted UF columns. The results may suggest that in the investigated period the removal processes were independent of the bacterial sulphate reduction and that peat is not readily available for SRB as a source of organic carbon. The comparable results for the removal of Cu, Pb and Zn may result from the sufficient amount of adsorption sites both on the organic and mineral media. The increased removal of Ni by the UF-PP CWs could have been caused by lower affinity of gravel for Ni and saturation of gravel with this metal. In the FaD columns the difference between the removal in the gravel and peat columns was statistically significant for Cu and Zn. The removal of both Cu and Zn was markedly higher in the FaD columns with gravel (FaD-GP) by about 16% for Cu, and about 40% for Zn. For the other metals that were determined the difference in the removal

was less than 5% in the FaD columns. The more efficient removal of Cu and Zn in the FaD-GP columns might be attributed to the supposition that gravel promotes oxic processes more efficiently than peat, as oxic processes can occur in the initial stage of holding phase in the FaD columns. The other cause for the higher efficiency of the FaD-GP columns was that the FaD-PP column (as only one was in operation) had very low porosity and hydraulic permeability.

One of the drawbacks of using organic bed media is that elevated concentrations of organic matter may be present in the effluent. Tab. 7.14 presents the influent and effluent data for all the columns from the initial period of the experiment.

Table 7.14 Influent and effluent TOC (median ± MAD, mg/L) concentrations in weeks 1−17

TOC	**Effluent from column**						
mg/L	**UF-PP**	**UF-PU**	**UF-GP**	**UF-GU**	**FaD-PP**	**FaD-GP**	**Influent**
Median	18.35	13.74	6.96	7.32	126.60	5.24	3.58
MAD	16.59	13.04	6.75	7.10	130.10	5.01	1.79

It can be observed that TOC concentration was very low in the influent as it is often a case in the electroplating wastewater. The TOC concentration was increased in the effluent from all the columns. It was less than 2-fold higher in the effluent from the columns with mineral media (UF-GP, UF-GU and FaD-GP) and about 4- to 5-fold higher in the UF columns with peat (UF-PP and UF-PU). Markedly higher TOC concentration was observed in the effluent from the FaD-PP column and it was 35-fold higher than in the influent and about 7-fold higher that the second highest effluent concentration. This could result from long duration of the holding phase in the FaD columns but probably also from the mode of operation.

7.5.7 Effect of the external carbon source addition

The treatment of wastewater containing metals in anaerobic CWs is often based on the assumption that precipitation of metals with biogenic sulphides would be the predominant removal process. As it was already mentioned in this thesis the activity of the SRB depends on the availability of simple organic compounds, amongst other things. The source of these compounds may origin from the inside or the outside of the system. The former solution was applied when the experimental system was constructed by adding organic media to the bed media in system A (in which it was compost) and system B (in which peat was used). The latter source of organic carbon in the form of lactate was started to be added to the influent of the columns with mineral media in week 44. The behaviour of the UF-GP columns to which lactate

was started to be added in week 44 is depicted in Fig. 7.11 as effluent concentrations of sulphates and Mn in weeks 30−53 of the experiment

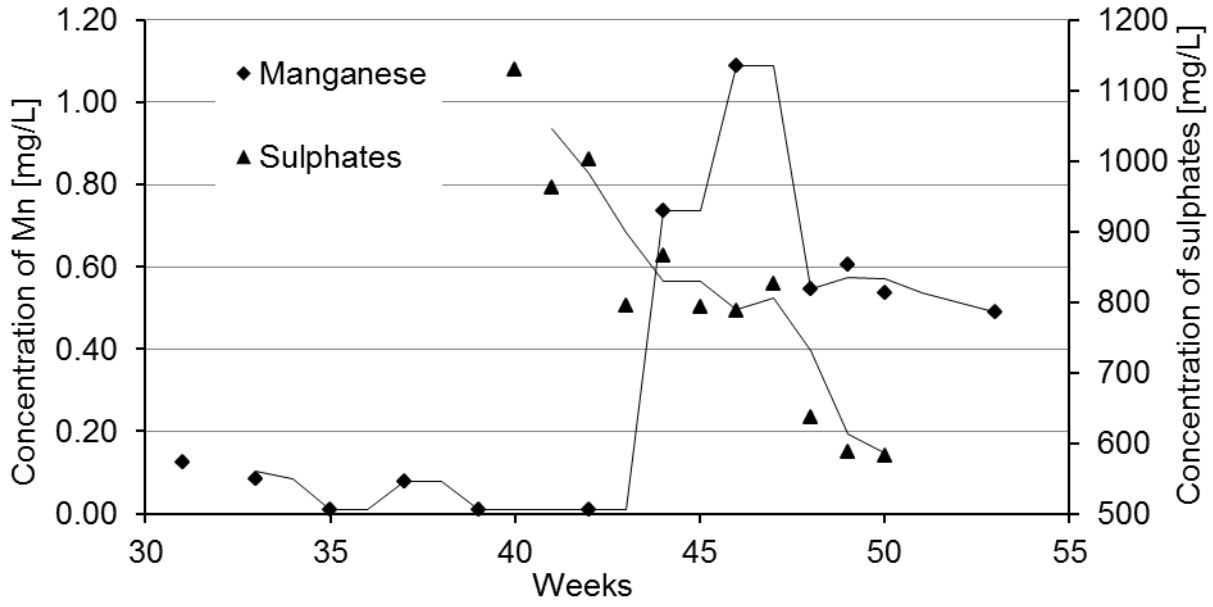


Figure 7.11 The concentration of manganese (Mn) and sulphates as a function of time in the UF-GP columns to which external carbon was started to be added in week 44 (weeks 31−53)

It can be observed that the sulphate concentration was decreasing in the whole period presented in Fig. 7.11. When the lactates were added the concentration of sulphates was in a pseudo steady-state and started decreasing after week 47 with a certain lag relative to the moment when the lactates were started to be added to the influent. The concentration of sulphates in the effluent decreased by more than 200 mg/L after the addition of lactates, taking the above-mentioned steady-state values as a reference. As it can be seen in Fig 7.11 also the effluent concentrations of Mn are shown because an interesting phenomenon was noticed. In week 44 when lactates were started to be added to the FaD-GP columns and in week 46 the effluent concentration of Mn increased markedly to reach a pseudo steady-state at about 0.6 mg/L. This phenomena could have been caused by microbial reduction of oxidized forms of Mn, which was stimulated by the organic carbon that is required by the bacteria. Also, oxidized form of Mn could have been reduced in abiotic reactions with sulphides. Both phenomena were discussed in Section 5.1.3

For comparison the same type of data as presented in Fig. 7.11 are given in Fig. 7.12 to describe the behaviour of the UF-PP columns to which no external carbon source was added.

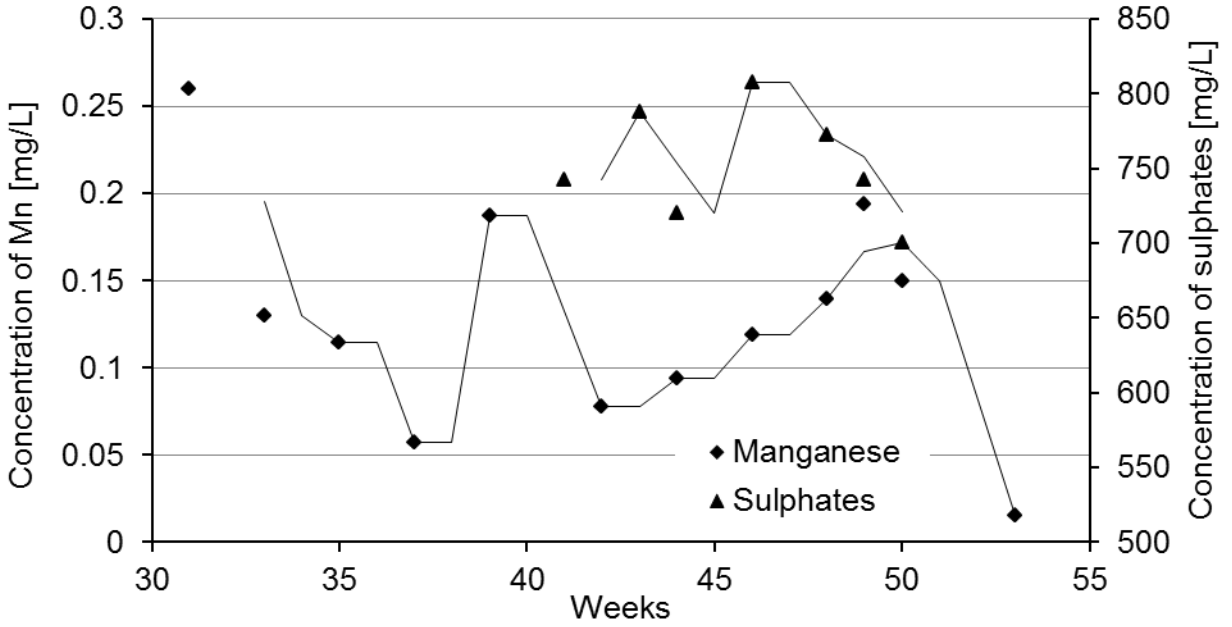


Figure 7.12 The concentration of manganese (Mn) and sulphates as a function of time in the UF-PP columns to which external carbon was not added (weeks 31-53)

The behaviour of the UF-PP colums, to which the external carbon source was not added, did not follow the observations for the UF-GP columns (Fig. 7.11). The decrease of the effluent sulphate concentration was also observed but it was not as marked as in the UF-GP columns. Also, the effluent concentration of Mn did not increase sharply in week 44 and on.

The main goal of adding the external carbon source to the influent was to enhance the removal of metals. The comparison of the metal removal efficiencies in the columns treating the influent with and without the external carbon source are given in Tab. 7.15 for weeks 44−53.

Table 7.15 Comparison between metal removal efficiencies (Median ± MAD, %) in columns treating wastewater with external carbon source (ECS) and without external carbon source (No ECS) external carbon source (weeks 44−53, n = 5−8)

	UF columns		**FaD columns**	
	UF-PP	**UF-GP**	**FaD-PP**	**FaD-GP**
	No ECS	**ECS**	**No ECS**	**ECS**
Cu	96.0±1.3	98.1±0.6	58.0±12.7	93.0±1.9
Ni	98.1±0.8	97.8±0.3	93.7±1.3	83.9±6.6
Pb	**46.2±32.6**	**69.6±9.8**	54.7±15.3	71.1±7.8
Zn	97.1±0.6	93.5±3.0	**73.7±5.5**	**92.6±4.8**
$\mathbf{SO_4^{2-}}$	10.7±3.5	4.0±21.9	24.3±21.1	16.2±12.1

Statistically significant differences are shown in bold

The results given in Tab. 7.15 suggest that the addition of the external carbon source may allow treatment with comparable metal removal efficiency. It can be seen in Tab. 7.15 that statistically significant differences between the removal efficiencies were only for Pb and Zn, which were

removed about 20% more efficiently in the UF-GP and FaD-GP columns, respectively, than in the peat columns. The other differences, which are not statistically significant, are markedly different, in terms of values, only between the FaD columns. The removal of sulphates is higher in the columns with peat, but the removal is low in all the types of columns presented in Tab. 7.15.

The data presented in Tab. 7.15 are of particular interest when the design decisions are to be made, but they furnish no information if the treatment efficiency was improved by the addition of the external carbon source. This information can be obtained, for example, by comparing the data in Tab. 7.15 and Tab. 7.13. More direct comparison of the treatment efficiency before and after the addition of the external carbon source is presented in Tab. 7.16 for weeks 45-53, this is with one-week lag relative to the moment when lactates were started to be added to the influent, and for weeks 33-44 when lactates were not added.

Table 7.16 Comparison between metal removal efficiencies (Median ± MAD, %) before (weeks 33−44, n = 5−8) and after addition of external carbon source (ECS) (weeks 45−53, n = 4−7)

	UF-GP		**FaD-GP**	
	No ECS	**ECS**	**No ECS**	**ECS**
Cu	**94.9±6.1**	**98.2±0.5**	90.67±6.2	93.6±2.1
Ni	**66.1±4.2**	**97.9±0.2**	80.9±3.5	88.9±6.1
Pb	52.0±21.2	69.5±21.5	50.2±28.5	71.5±7.8
Zn	**74.0±11.2**	**93.9±1.8**	83.7±6.5	93.1±3.1
$\mathbf{SO_4^{2-}}$	**-24.1±6.1**	**4.0±21.9**	-2.3±2.6	16.2±12.1

Statistically significant differences are shown in bold

The addition of the external carbon source improved the removal efficiency of metals and sulphates as shown in Tab. 7.16. The statistically significant differences were observed for Cu, Ni, Zn and sulphates in the UF-GP columns. The most pronounced improvement of the treatment efficiency was obtained for Ni (by about 32%) in the UF-GP columns. Only the removal efficiency of Cu in both UF-GP and FaD-GP columns increased by less than 4%. The other removal improvements were higher. The removal of sulphates was higher in the FaD-GP columns, which can be attributed to the longer wastewater retention time

One of the drawbacks of using external carbon sources is that it increases the COD of the wastewater. This issue, however, must not be considered without comparing the effect of external and internal carbon sourced as a cause for residual COD in the effluent. The other aspect that should be addressed is the release of reduced sulphur species from the system. They are more toxic to the environment than sulphate and may cause odour nuisance in some cases. The

influent and effluent COD and concentrations and the corresponding removal are shown in Tab. 7.17. Also the ratio COD/SO_4^{2-} in the influent is given as it is an essential parameter for the bacterial sulphate reduction.

Table 7.17 COD and sulphates removal in the experimental system in weeks 44-49

	COD		**SO_4^{2-}**		**Influent COD/SO_4^{2-} ratio**
	Concentration[1] [mg O_2/L]	**Removal[2] [%]**	**Concentration[1] [mg/L]**	**Removal[2] [%]**	
Influent	32±2		860±47.5		0.04
Effluent					
UF-PP	94±6	-197.0	742.5±36.25	13.7	
UF-PU	76±19	-140.2	720±13.75	16.3	
FaD-PP	217±0	-585.3	710±130	17.4	
Influent+ECS[3]	273±21		855±40		0.32
Effluent					
UF-GP	69±11	74.6	775±70	9.4	
UF-GU	75±11	72.7	615±55	28.1	
FaD-GP	104±28	61.8	746.25±97.5	12.7	

[1]median±MAD, [2]calculated based on the median values for the influent and effluent; n = 4−6 for COD, except n = 1 for the FaD-PP column; n = 5−9 for sulphates; data for weeks 44−49, [3]external carbon source

The results in Tab. 7.17 show that, even though, COD of the wastewater with the external carbon source was about 9-fold higher than the influent COD of the wastewater without the external carbon source the effluent COD of the former wastewater is lower than for the latter. The maximum allowable concentration according to the Regulation by the Polish Ministry of the Environment is 125 mg O_2/L (PL MoE, 2006) and it was exceeded only in the FaD-PP column, which was the least efficient in many respects. Also the effluent concentrations of sulphates in all the types of columns are comparable, with the exception of the UF-GU columns, which appeared particularly efficient supposedly due to the lack of oxygenated root zone. Taking into account the influent and effluent concentration of sulphates it can be assumed that there is a possibility that reduced species may appear in the effluent in comparable amounts from the columns with the internal and external carbon source. It should also be mentioned that some of the reduced sulphur species were probably oxidized to elemental sulphur in the outflow zone of the columns, which was observed and analysed (Section 7.8.8). The ratio COD/SO_4^{2-} in the influent with the external carbon source was 0.32, this is, near the intended value of 0.3, which was proved optimum for the bacterial sulphate reduction as reported by Song et al. (1998) and is discussed in Section 5.4.3. This ratio was much lower for the influent without the external carbon source, namely 0.04, however, the reduction of sulphates occurred as the organic-carbon source was in the media, so the actual ratio is unknown.

Noteworthy, no steady state was reached for the removal of metals after the addition of the external carbon source as it was indicated in (Fig. 7.6−7.9). This suggests that the treatment efficiency was still increasing making the external carbon source a reasonable alternative to the internal carbon source used in the experiment.

7.6 Sorption properties of the substrates

The sorption properties of sand and compost (mixture with sand), which were used as bed media in system A, were assessed in laboratory conditions using solutions spiked with increasing metal and boron concentrations. The obtained data were well described by the Langmuir adsorption model (Section 6.6). The obtained Langmuir adsorption maxima (X_m, mg/kg) are given in Tab. 7.18.

Table 7.18 Langmuir adsorption maxima (X_m, mg/kg DW) of Al, Cu, Ni, Zn for sand and compost (1:1 by vol. with sand), and maximum adsorbed B concentrations, system A ($R^2 \geq 0.79$)

	Al	**B**	**Cu**	**Ni**	**Zn**
Sand	25.25	22.87	38.41	8.38	21.58
Compost (mixture)	694.18	74.91	818.19	176.62	148.78

The Langmuir adsorption maxima presented in Tab. 7.18 indicate that the compost used in system A had much higher sorption capacity than sand, especially for metals, as for B the capacity of compost was only about three times higher. The capacity of compost was 21- to 27-fold higher than of sand for Al, Cu and Ni, and only 7-fold for Zn. The highest capacity was observed for Cu both in compost and sand, which corresponds well to the observed high removal of Cu in system A (Tab. 7.1 and 7.2).

Based on the calculated adsorption maxima for metals and B it is possible to calculate a theoretical longevity of the sand and compost columns using the concentrations of these elements in various types of wastewater used in this study, both in system A and B. In Tab. 7.19 the theoretical longevity of the sand and compost columns is given with the corresponding influent concentrations of metals and B in all the types of influent used in system A and B. The removal of Al and B was not studied in system B, but in Tab. 7.19 their concentrations were assumed to be at the same level as for the other elements, for the sake of comparison. The assumptions of these calculations were that 100% removal is obtained and that dead volumes are absent in the columns and also that the inflow rate is 3.25 mL/min, which was applied to the UF columns in system A and system B (before week 33). The longevity of the FaD columns with two-week holding phase would be about 6.5-fold higher.

Table 7.19 Theoretical longevity of the sand and mixed sand-compost bed (compost) media depending on the type of influent at the inflow rate of 3.25 mL/min

Type of influent	Parameter	Al	B	Cu	Ni	Zn
Feed without cyanides (A)	Influent					
	conc., mg/L	0.264	5.43	0.095	0.058	0.022
	Sand longevity, d	657	29	2778	993	2617
	Compost longevity, d	16856	88	55209	19520	43351
Cyanide wastewater (A)	Influent					
	conc., mg/L	2.768	4.03	2.315	0.037	0.015
	Sand longevity, d	63	39	114	1556	3838
	Compost longevity, d	1608	119	2266	30599	63581
Low-strength influent (B)	Influent					
	conc., mg/L	1.00[1]	1.00[1]	1.00	1.00	1.00
	Sand longevity, d	173	157	264	58	58
	Compost longevity, d	4450	480	5245	1132	954
High-strength influent (B)	Influent					
	conc., mg/L	5.00[1]	5.00[1]	5.00	5.00	5.00
	Sand longevity, d	35	31	53	12	12
	Compost longevity, d	890	96	1049	226	191
Raw wastewater	Influent					
	conc., mg/L	75.11	-	39.32	31.70	3.48
	Sand longevity, d	2	-	7	2	17
	Compost longevity, d	59	-	133	36	274

[1]Al and B were not added to the influents of system B, these values were assumed for the sake of comparison

It can be seen in Tab. 7.19 that the predicted longevity of the sand and compost columns of system A does not match the observed performance relative to all the elements (Tab. 7.19, and 7.1 and 7.2). In particular, high sorption capacity of both sand and compost for Ni and Zn does not reflect the behaviour of these metals in the FaD columns. Zn was released from the FaD columns of system A, and Ni was released from the FaD-SP columns fed with the cyanide wastewater. For the other elements the predicted longevity is more consistent with performance data. The removal of Al and Cu was invariably high in all the types of FaD columns. The longevity of the sand columns for Al seems low, but the value of 63 d should be multiplied by 6.5 to obtain the longevity of the sand column operated in two-week batch mode. Also the cyanide wastewater was only fed to system A for about 12 weeks. The longevity of the sand and columns for B are in agreement with the performance data. The longevity of the FaD-SP columns for B is about 190 d (29d ^{x} 6.5) when the feed without cyanides is treated. The first measurements were taken after 300 days of the experiment after, theoretically, these columns were saturated with B. The median value for the removal of B in the FaD-SP columns is 1.4%, which is in agreement with the predicted longevity of these columns. The removal of B in the

FaD-CP columns was 37.7% as the predicted longevity is 572 d, which is longer than the duration of the experiment.

The results of the sorption experiment as presented in Tab. 7.18 and 7.19 can be a valuable information for the design of a CW system. There is, however, little agreement between the predictions and the performance of the system. This may be due to several factors. The conditions in the treatment system and in the sorption experiment are different in terms of wastewater composition, pH, ORP and others. The changing oxic and anoxic conditions in the FaD columns could have attenuated the adsorption process or even cause desorption of metals and B. There are several others factors that can be responsible for desorption. Adsorption is not the only process occurring in the experimental columns, thus none of the phenomena observed based on the comparison of the influent-effluent data cannot be directly related to adsorption without any additional analyses. Also the presence of dead volumes may cause that the actual longevity would be lower than the expected one. Propensity for desorption of metals can be assessed, amongst others, by the sequential extraction procedure, as described in Section 7.7.

7.7 Metal and sulphur concentration and fractions in the substrate

The fractionation of metals in the substrate of CWs is highly important as unstable forms of metals may be easily leached from the system. The substrates from systems A and B were analysed by the SEP to elucidate the fractionation of metals (and S) in the experimental systems.

7.7.1 Fractions and concentration of contaminants in the substrate from system A

The SEP was performed for the substrates sampled from the top and bottom of the columns in system A after cessation of the experiment. The presented results are for the FaD columns fed with the cyanide wastewater, for the FaD-CP column in Fig. 7.13 and for the FaD-SP column in Fig. 7.14.

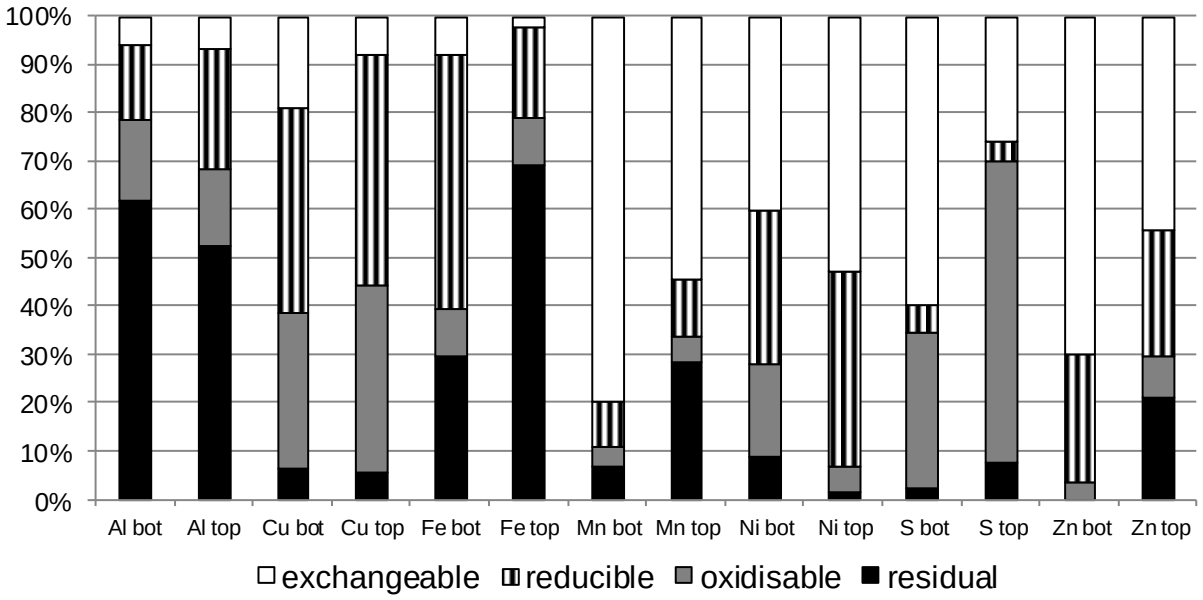


Figure 7.13 Metal and sulphur fractions (by SEP) in top and bottom (bot.) substrate layers of the FaD-CP column fed with the cyanide wastewater, system A

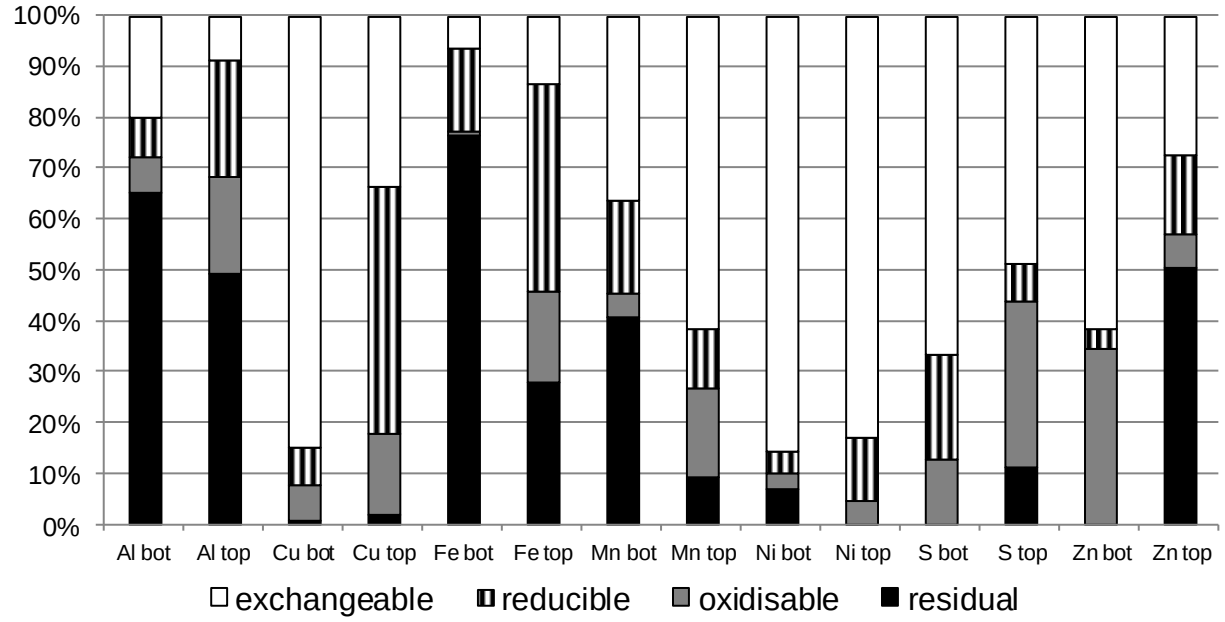


Figure 7.14 Metal and sulphur fractions (by SEP) in top and bottom (bot.) substrate layers of the FaD-SP column fed with the cyanide wastewater, system A

Aluminium was bound mostly within the residual fraction, which ensures its stability in substrate. However, most of the residual fraction could have originated from the raw material either sand or compost. It was observed that the reducible fraction was higher in the top of substrate, probably due to the effect of rhizosphere. Copper was bound to more stable fractions, with the exception of the bottom of the FaD-SP column where most of Cu was bound with

exchangeable fraction. This ‘strong binding’ of Al and Cu corresponds to their high removal in system A. The results presented in Fig. 7.13 and 7.14 show that most of Mn, Zn and Ni was present in exchangeable fraction which can be easily released from the columns filled with either organic and mineral media. These findings are in agreement with the results presented in Tab. 7.2, which show that these elements were released from all the FaD columns fed with the cyanide wastewater. It should be noted that the exchangeable fraction of metals and sulphur is generally higher in the substrate of the FaD-SP column. The exception is the fractionation of Zn and Mn. This may stem from the fact that the exchangeable fraction of Mn and Zn was higher in compost than in sand as shown in Fig. 7.15.

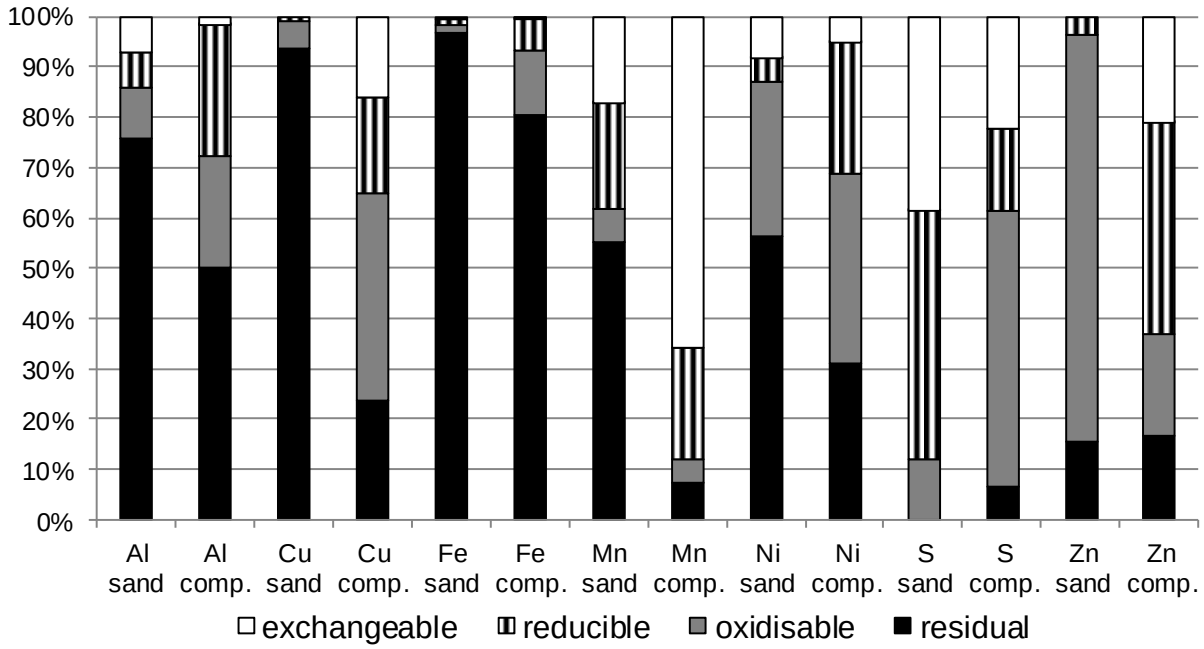


Figure 7.15 Fractionantion of metals and sulphur in the raw substrates: sand and compost (comp.)

When the fractionation of metals and sulphur in the substrate from the FaD-CP column and the raw compost were compared it was observed that: (i) the exchangeable fraction of Al increased slightly and also that the residual fraction increased; (ii) for Cu the reducible fraction increased and residual fraction diminished; (iii) the residual fraction of Fe diminished markedly in the bottom part (by ca. 50%) and slightly in the top part (by ca. 10%), and the reducible fraction (less than 10% in the raw material) increased (by 50%) in the bottom layer and by cc. 20% in the top layer; (iv) the Mn exchangeable fraction increased by ca. 15% in the bottom part, and decreased by 10% for the residual fraction; (v) the Ni residual fraction decreased by more than 20%, and the exchangeable fraction increased by more than 30%; (vi) for S the decrease of the oxidisable fraction was observed, and the increase of the exchangeable fraction by more than

30% in the bottom part and less than 5% in the top part, (vii) the increase of the residual fraction of Zn in the top part by less than 5% was observed and the increase of the exchangeable fraction by less than 20%, in the bottom part of the column the exchangeable fraction of Zn increased by almost 50% and the residual fraction was approx. 0%.

When the fractionation of metals and sulphur in the substrate from the FaD-SP column and the raw compost were compared it was observed that: (i) the exchangeable fraction of Al increased slightly by about 15% in the bottom part and by less than 5% in the top part of the column, the residual fraction decreased by about 10% and 25% for the bottom and the top, respectively, the residual fraction is less than 5% in both top and bottom of the column, and the exchangeable fraction increased by about 85% in the bottom part and by about 35% in the top part, (ii) for Fe and Mn the residual fraction decreased more in the top parts, (iii) for Ni the residual fraction decreased by about 50%, (iv) the exchangeable fraction of S increased by at least about 20% and the reducible fraction diminished proportionally in the bottom part, whereas in the top part the residual and oxidisable fraction increased markedly and the exchangeable fraction increased by 10%; (v) the residual fraction of Zn in the top part of the column increased by about 35% and the exchangeable fraction increased by ca. 25% and in the bottom part the exchangeable fraction of Zn increased by 60% and the oxidisable fraction decreased proportionally, so that the residual fraction was practically completely washed out.

The amount of metals and sulphur extracted in the single-step *aqua regia* digestion was assumed to represent a pseudo-total concentration of these elements in the substrates (Tab. 7.20). *Aqua regia* is not able to liberate the metals that are silicate-bound, but the use of this reagent to give a 'pseudo-total' analysis of the substrate is acceptable in this case as the silicate fraction was not of interest in this study (Dean, 2003).

Tab. 7.20 Pseudo-total concentration of metals (mg/kg DW) in the bottom and top layers of substrate sampled from the FaD columns fed with the cyanide wastewater (system A) and in the raw materials (sand and compost-sand mixture)

Column	Sample location	Al	Cu	Fe	Mn	Ni	S	Zn
FaD-CP	**bottom**	111.226	1.129	260.833	6.017	0.626	293.193	4.090
	top	846.514	70.966	4577.979	33.686	23.131	865.416	15.430
FaD-SP	**bottom**	28.638	2.806	129.453	0.917	1.126	88.987	1.466
	top	155.812	58.161	2852.230	7.116	17.616	358.218	7.499
Compost[1]	**Raw material**	1113.083	2.450	1186.932	42.797	2.260	211.149	9.222
Sand	**Raw material**	148.259	0.018	1464.720	4.834	1.245	17.367	4.565

[1]compost-sand mixture

Generally, the concentration of metals and S is higher in the substrate of the FaD-CP column than in the FaD-SP column. This is because the initial concentration of these elements was higher in the compost than in the sand. This observation is not viable for all the metals, for example, for Fe, and Ni and Cu. The initial and the final concentration of the elements allows for observation if they were released or stored in the substrate. The release of the element might be attributed to its mobilization and subsequent fate such as wash-out from the system, uptake by plants or removal in the down-stream parts of the column. In Tab. 7.20 it can be seen that all the elements, save S and Cu, were leached to some extent from the bottom part of the FaD columns. Most of the elements (in terms of concentration) were accumulated in the top part of the FaD columns. The exception was Al and Mn in the FaD-CP, which were leached from the top part of this column. These differences between the top and bottom parts of the FaD columns may result probably from the fact that the influent was fed from the top, therefore major particulate fraction of the elements was entrapped in the top part of the FaD columns. The dense system of rhizomes and roots in the upper part of the columns was presumably playing a major role in filtering out the contaminants and further removal in the oxidized root zone. The soluble fraction of contaminants was probably adsorbed on the upper part of the column, either the substrate or the plant biomass, and could have been precipitated as sulphides or oxy(hydroxides), depending on the ORP.

7.7.2 Comparison of metal fractionation and concentration in the substrates in systems A and B

The UF columns were the other type of CW studied in the experiment. It was assumed that the process conditions different to those occurring in the FaD columns would results in different performance but also distribution of the contaminants in the columns. The upward flow direction and constant saturation of the bed media was believed, when designing the experiment, to promote stable anoxic conditions and to remove majority of the contaminants in the bottom part of the column. To verify this hypothesis the bed media from the planted UF columns were analysed by the SEP and also the SEM-EDS method. The results of the SEP of the bottom and top parts of the UF-CP, UF-SP columns (both system A) and the UF-PP and UF-GP columns (both system B), are presented in Fig. 7.16−7.19, respectively. The elements presented for the UF columns of system A are: Cu, Ni, Zn; and for the UF columns of system B: Cu, Ni and Pb.

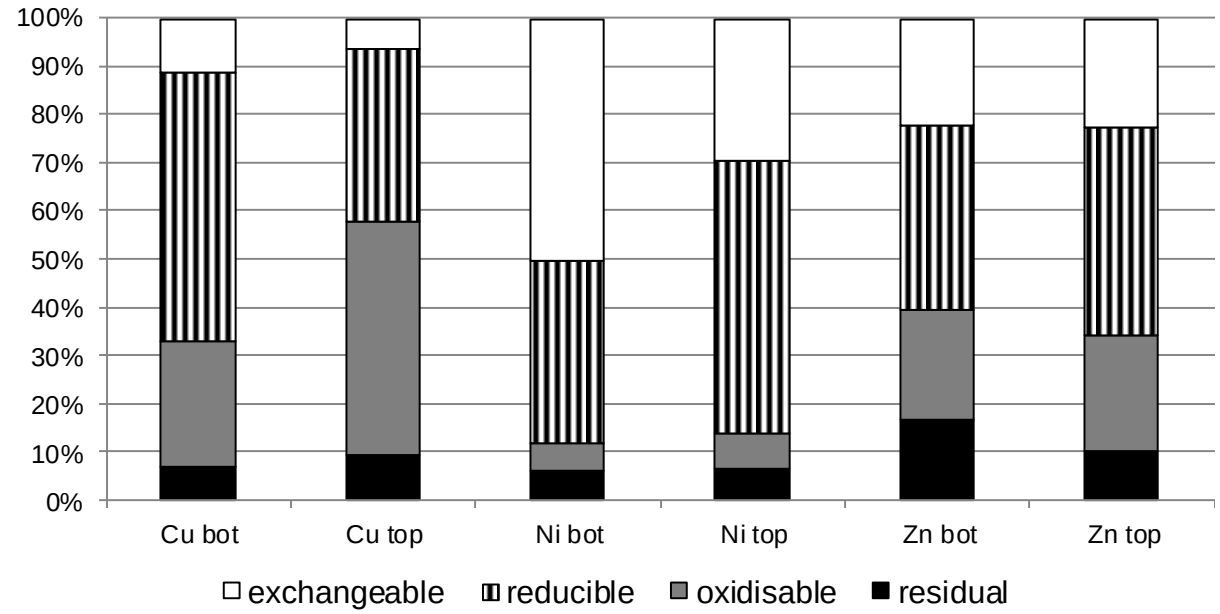


Figure 7.16 Metal fractions in the UF-CP column (cyanide wastewater), system A

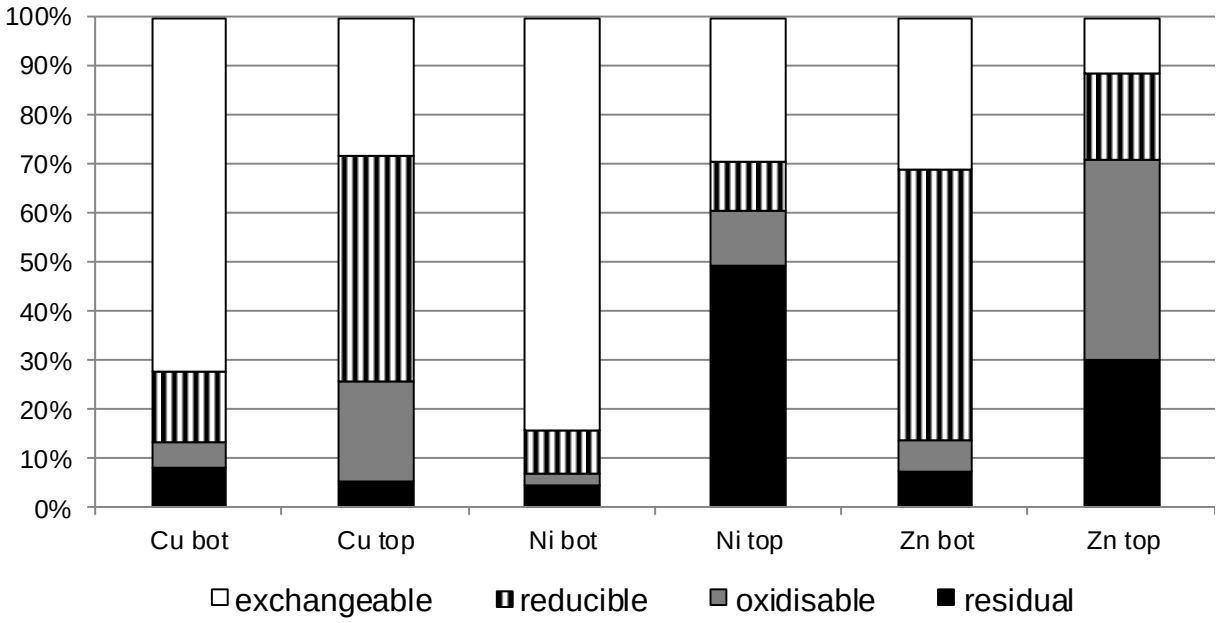


Figure 7.17 Metal fractions in the UF-SP column (cyanide wastewater), system A

The observed differences between for the Cu concentration in the UF-CP and UF-SP columns in system A are: small exchangeable fraction in the UF-CP columns compared to large fraction in the bottom part of the UF-SP column (more than 70%) and moderate fraction in the top part of these columns (about 30%). For Ni in the bottom layer the exchangeable fraction constitutes 50% and more than 80% of the sum of the fractions in the UF-CP and UF-SP columns, respectively; and ca. 30% in the top layers in both columns. The largest fraction of Ni in the top part of the UF-CP columns is the reducible one and in the UF-SP is the residual one (similar to

the contribution of this fraction in the raw sand); the exchangeable fraction of Zn is comparable in both types of the columns, the reducible fraction in the bottom part of the column is larger in UF-CP column, and the residual and oxidisable fraction in the top part of the columns is larger in the UF-SP column.

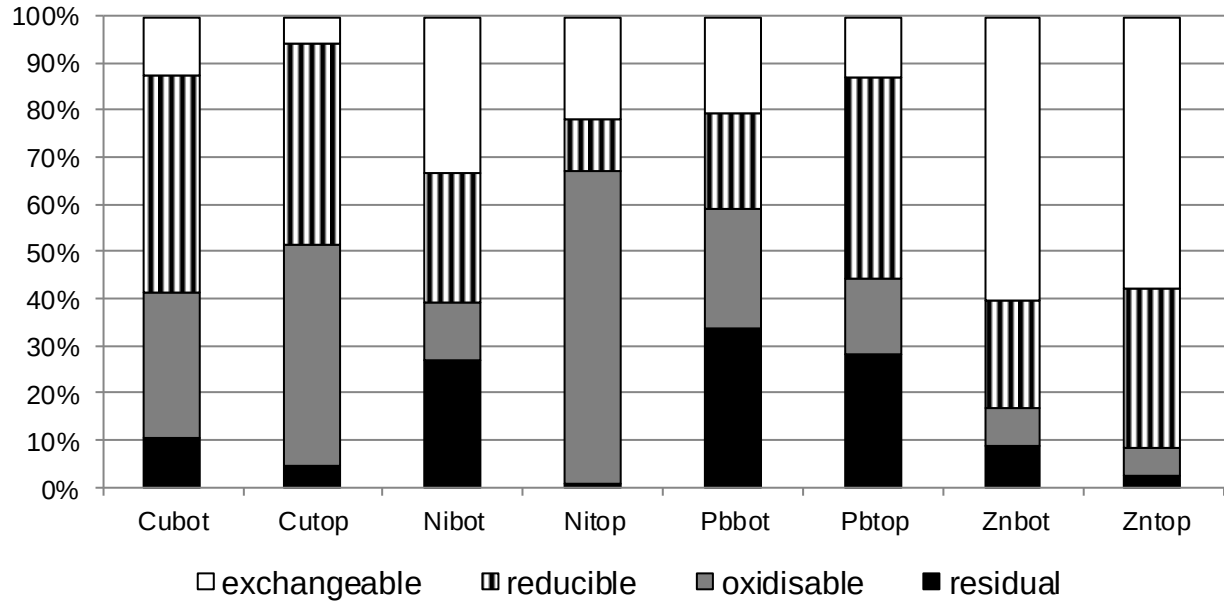


Figure 7.18 Metal fractions in the UF-PP column, system B

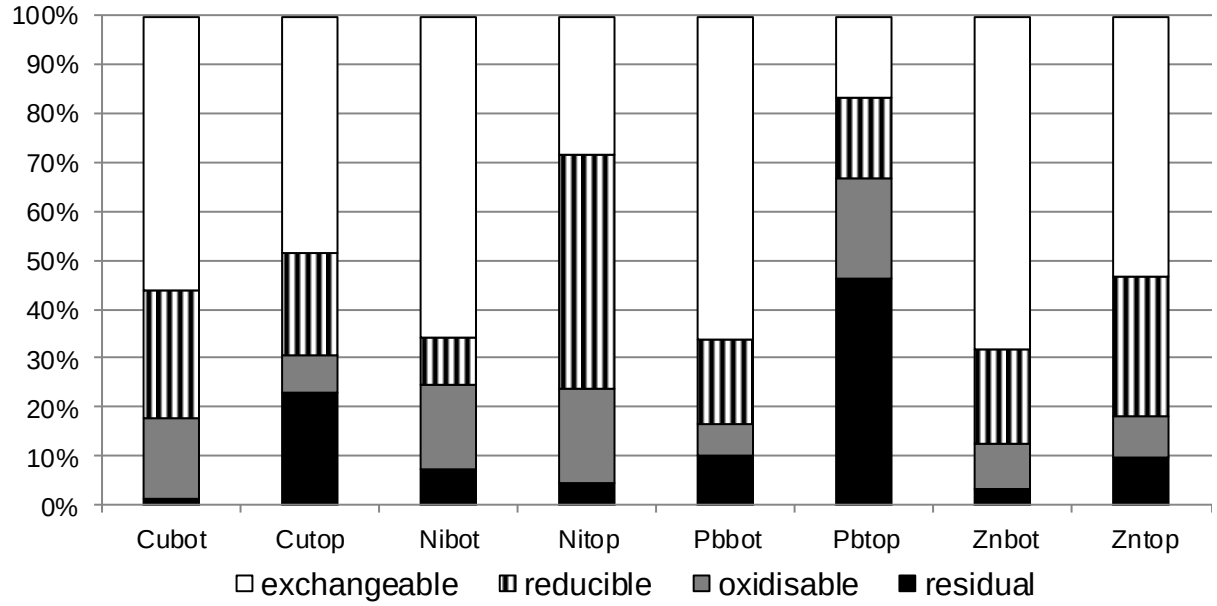


Figure 7.19 Metal fractions in the UF-GP column , system B

The observed differences between the UF-PP and UF-GP columns in system B are in general similar to the difference between the UF-CP and UF-SP columns in system A with several

exceptions, that Ni was bound to the substrate in more stable forms (residual and oxidisable) in the UF-PP column than in the UF-GP column. For Pb, as for the other metals, the exchangeable fraction is larger in the UF-GP columns, however, Pb was bound mostly in the residual and oxidisable fraction in the top part of the UF-GP columns, whereas in the UF-PP column it was mostly the exchangeable and reducible fraction. Conversely, in the top of the UF-PP column the residual and oxidisable fractions contributed the most to the binding of Pb, whereas in the UF-GP columns it was mainly the exchangeable fraction that Pb was bound to.

When the UF-PP (system B) and UF-CP (system A) columns were compared the following observations could be made: (i) similar pattern for binding of Cu: small exchangeable fraction in both top and bottom part of the column, reducible fraction is the largest in the bottom part and the oxidisable fraction is the largest in the top part; (ii) the exchangeable fraction of Ni was 10−15% larger in the UF-CP column, the exchangeable fraction of Ni was the largest in the bottom part and the reducible one in the top part of the UF-CP columns, whereas in the UF-PP column the oxidisable and residual fractions were much larger; (iii) in the UF-CP columns Zn was mostly bound to reducible fraction and comparable proportion of the exchangeable and residual fractions, whereas in the UF-PP column most of Zn was bound to the exchangeable fraction (about 60%) and the second largest reducible fraction.

When the UF-SP (system A) and UF-GP (system B) columns were compared the following observations could be made: (i) the exchangeable fraction is the dominant form of Cu in the bottom part of the UF-SP and the UF-GP columns, ca. 70% and 55%, respectively; the second largest fraction is the reducible one; in the bottom part of the UF-SP column the exchangeable fraction contributed the most to the binding of Cu, whereas in the UF-GP columns it was the exchangeable fraction and about 20% of the reducible and residual fraction, each; (ii) Ni was bound mostly in the exchangeable fraction in the bottom part of both columns, the exchangeable fraction of Ni was about 30% in both columns, but the largest fraction was the residual one in the UF-SP column and the reducible one in the UF-GP column; (iii) Zn was bound mostly (50−70%) in the exchangeable fraction in the UF-GP column, whereas the largest fraction in the bottom part of the UF-SP column was the reducible one, and in the top part the residual and oxidisable fraction constituted about 70% of the bound Zn.

The relative content of the metal fractions does not indicate the quantitative distribution in the columns and the concentration which in concert with speciation can furnish complete information on the toxicological risk associated with the contaminants bound in the CWs. The pseudo-total concentration of metals in the tested substrates is represented by the amount of

metals extracted in the *aqua regia* digestion of the substrate (Tab. 7.21). It should be noted that the pseudo-total concentration does not inform about the potential toxicity of the substrate, however, the concentration of the operationally-defined fractions can be easily calculated using the relative content as presented in Fig. 7.16−7.19. The presentation of the absolute content of the fractions was omitted in this thesis, as large difference between the concentrations of metals would diminish the clarity of the data presentation regarding the fractionation of metals.

Table 7.21 Pseudo-total concentration of metals (mg/kg DW) in the bottom and top layers of substrate sampled from the planted UF columns fed with the cyanide wastewater and the high-strength wastewater (systems A and B, respectively)

Column	Sample location	Cu	Ni	Pb	Zn	Fe	Mn
		System B, high-strength wastewater					
UF-PP	**bottom**	5.61	12.10	2.40	24.20	n.a.	n.a.
	top	11.74	66.65	7.47	103.9	n.a.	n.a.
UF-GP	**bottom**	59.91	91.1	20.14	132.61	n.a.	n.a.
	top	9.00	24.13	3.54	29.89	n.a.	n.a.
		System A					
		Feed without cyanides					
UF-CP	**bottom**	33.008	16.211	n.a.	13.530	2172.215	17.248
	top	3.518	3.048	n.a.	6.666	2134.049	24.704
UF-SP	**bottom**	4.076	1.249	n.a.	0.748	581.282	1.144
	top	1.170	1.331	n.a.	2.065	1793.104	2.900
		Cyanide wastewater					
UF-CP	**bottom**	57.922	8.742	n.a.	6.186	329.847	4.392
	top	28.968	8.912	n.a.	6.250	905.329	15.451
UF-SP	**bottom**	47.512	7.548	n.a.	3.442	2647.335	5.479
	top	8.791	2.642	n.a.	4.674	2222.252	17.196

n.a. – not analysed

In system B the main difference between the UF-PP and UF-GP columns consisted in the quantitative distribution of metals. In the UF-PP columns higher concentration of all the metals was observed in the top part of the columns in contrast to the UF-GP column. It is difficult to elucidate the cause of different behaviour of metals in the UF-PP and the UF-GP columns. The probable explanation could have been the acidifying effect of peat that enhanced mobility of metals entering the system and deeper penetration into the column with subsequent binding in the top part of the column at the interface between anoxic and oxic layers where various processes could play a role in the removal. The concentration of metals in both parts of the planted UF columns of system B was in the decreasing order of Zn > Ni> Cu > Pb.

The findings for the distribution of metals in the planted UF columns of system A were slightly different compared to system B. The concentration of Cu was invariably higher in the bottom

part of the columns. The content of Ni was distributed in a manner independent of the strength of wastewater and the type of substrate. Zn was evenly distributed in the UF columns fed with the cyanide wastewater, and in the columns fed with the feed without cyanides the concentration of Zn was higher in the bottom part of the UF-CP column, and conversely in the UF-SP column. The distribution of Fe and Mn seemed to follow a different pattern. In general, larger portion of these metals was bound in the top parts of the columns. This behaviour can probably be attributed to the effect of rhizomes and roots of the plants, which promote oxidation of Fe and Mn, and formation of a plaque on the belowground biomass of the plants. The data given in Tab. 7.21 may also suggest that Zn co-precipitated with Fe and Mn in the top part of the planted UF columns.

It can be seen that the concentration of Cu, Ni and Zn in the substrates of system A and B are comparable and often of the same order of magnitude. The concentration of Ni and Zn is higher in the substrates of system B, as the influent concentrations were higher. The Cu concentration is comparable between system B and system A (for the high-strength influent and the cyanide wastewater, respectively).

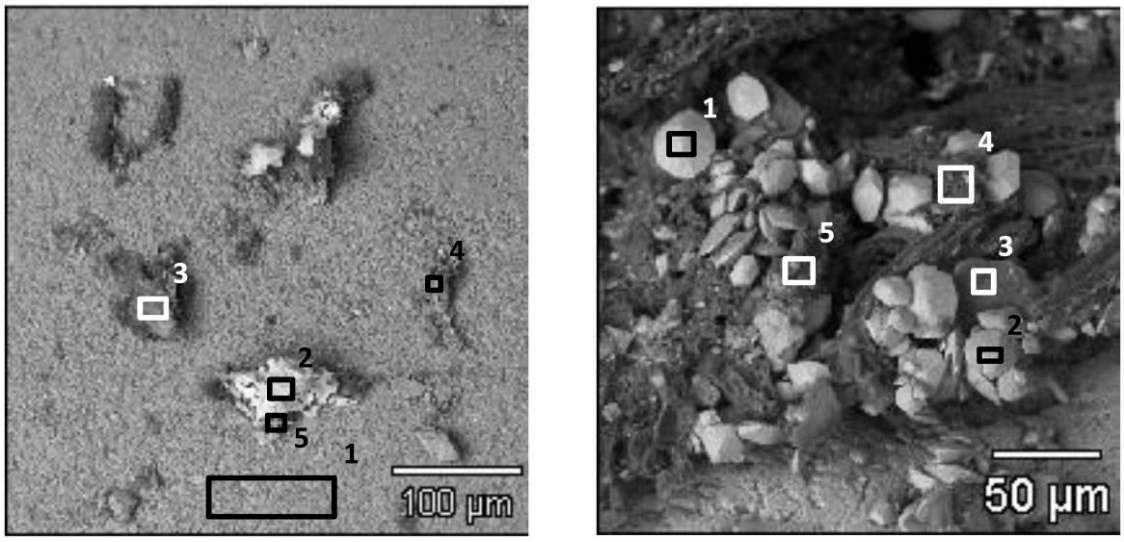
7.8 SEM-EDS analysis of the substrates from systems A and B

The applicability of chemical analysis for anoxic sediments has been widely discussed in the literature. It was suggested that commonly applied sequential extraction protocols may underestimate the amount of sulphides. The chemical analyses of anoxic sediments should be evaluated using X-ray absorption spectroscopy or SEM-EDS (Peltier et al., 2005). In this study the latter method was used mainly to study the surface of the substrate samples for which the SEP was performed in system A and B, with some additional samples for the substrate sampled from the columns of system B that were not analysed by the sequential chemical extraction. SEM-EDS analysis was also carried out to analyse the gravel on the very surface of the FaD-GU column, in which the presence of elemental sulphur was suspected. Additionally, the surface of the filters used to filter the effluent from the UF-PP and the FaD-GU columns were subjected to the SEM-EDS analysis.

7.8.1 SEM-EDS analysis of the substrate from the FaD-CP column (system A)

The substrate from the FaD-CP column fed with the cyanide wastewater was analysed by the SEM-EDS method taking into account the location of sample: bottom and top of the column. The results of the SEM-EDS analysis of the substrate sampled from the bottom of the column are shown in Fig. 7.20 and in Tab. 7.22. Figure 7.20 presents two SEM-BSE images of the sample

with numbered areas analysed by EDS. Table 7.22 shows the results of the EDS analyses of the areas indicated in Fig. 7.20. The samples in Tab. 7.22 are named according to the name of a sample written below the images in Fig. 7.20. The names of the samples include the name of a column and sampling spot (top or bottom). In Tab. 7.22 the areas for the left-hand image (image a) were listed first. The results for the other samples will be presented in the same manner. The colours of the rectangles indicating the selected area may be ignored. White or black colours were used to increase visibility.



(a) Sample FaD-CP-bot(1) (b) Sample FaD-CP-bot(2)

Figure 7.20 SEM-BSE images of wetland substrates sampled from the bottom (bot) of the FaD-CP column fed with the cyanide wastewater (system A)

Table 7.22 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.20 determined using EDS

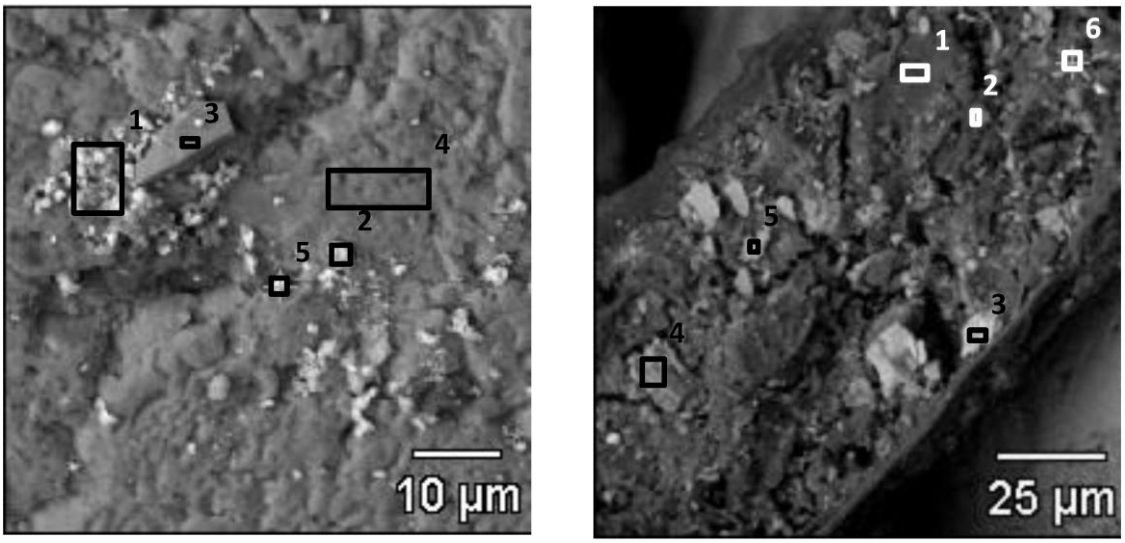
Sample_Area	**Atomic %**[1]											
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**Cl**	**K**	**Ca**	**Ti**	**Mn**	**Fe**
FaD-CP-bot(1)_ar1	-	-	1.9	98.1	-	-	-	-	-	-	-	-
FaD-CP-bot(1)_ar2	-	-	2.1	49.6	0.4	0.7	-	1.1	0.5	-	42.2	3.3
FaD-CP-bot(1)_ar3	1.7	0.7	7.6	75.7	2.0	2.7	-	1.3	0.7	0.5		7.2
FaD-CP-bot(1)_ar4	2.5	-	8.5	58.0	1.7	2.7	-	1.1	0.8	-	17.8	6.9
FaD-CP-bot(1)_ar5	3.4	0.7	5.6	73.0	1.1	3.5	-	1.3	1.8	-	5.3	4.3
FaD-CP-bot(2)_ar1	1.2	0.2	1.0	11.8	-	44.1	-		41.0	-	-	0.7
FaD-CP-bot(2)_ar2	0.8	-	0.6	14.4	-	42.4	-	0.3	41.0	-	-	0.6
FaD-CP-bot(2)_ar3	9.7	1.9	4.2	21.3	-	31.8	0.9	2.7	24.6	0.4	-	2.4
FaD-CP-bot(2)_ar4	12.8	2.3	5.2	34.1	0.9	24.6	-	3.2	11.6	1.2	-	4.0
FaD-CP-bot(2)_ar5	11.9	2.5	9.9	40.0	2.1	14.8	0.6	3.4	5.5	0.8	-	8.5

[1]Compositions normalized to 100%

The FaD-CP-bot(1) sample contains mainly detrital matter associated with Al silicate and probably Fe-Mn oxy(hydroxides). The composition of area 1 of this sample corresponds to the composition of the sand used in this study (Tab. 7.33). The image of sample FaD-CP-bot(2)

presents a cluster consisting of several phases: calcium sulphates probably with detrital grains of quartz and other silica minerals. Also the presence of aluminium hydroxysulphate and carbonates cannot be excluded.

The SEM-BSE images of the samples taken from the top part of the FaD-CP column of system A are presented in Fig. 7.21 and the elemental composition of the selected areas are listed in Tab. 7.23.



(a) Sample FaD-CP-top(1) (b) Sample FaD-CP-top(2)

Figure 7.21 SEM-BSE images of wetland substrates sampled from the top of the FaD-CP column fed with the cyanide wastewater (system A)

Table 7.23 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.21 determined using EDS

Sample_Area	Atomic %[1]													
	Na	Mg	Al	Si	P	S	K	Ca	Mn	Fe	Ag	La	Ce	Nd
FaD-CP-top(1)_ar1	2.0	0.5	2.1	57.2	3.3	3.9	0.5	4.9	-	25.5	-	-	-	-
FaD-CP-top(1)_ar2	1.7	0.5	1.7	60.6	3.7	1.4	0.5	2.5	-	27.3	-	-	-	-
FaD-CP-top(1)_ar3	-	-	-	99.6	-	-	-	0.4	-	-	-	-	-	-
FaD-CP-top(1)_ar4	-	-	1.1	98.9	-	-	-	-	-	-	-	-	-	-
FaD-CP-top(1)_ar5	1.8	0.5	3.5	50.2	4.8	0.8	0.3	2.1	-	36.1	-	-	-	-
FaD-CP-top(2)_ar1	3.3	1.3	15.4	51.6	1.1	7.3	5.3	11.4	-	3.2	-	-	-	-
FaD-CP-top(2)_ar2	1.9	0.8	9.0	73.7	1.2	3.1	2.0	5.9	-	2.3	-	-	-	-
FaD-CP-top(2)_ar3	-	11.5	19.6	46.1	-	0.7	2.3	1.6	0.4	17.7	-	-	-	-
FaD-CP-top(2)_ar4	8.3	-	16.5	71.3	-	0.9	0.6	2.4	-		-	-	-	-
FaD-CP-top(2)_ar5	1.0	1.5	24.5	56.0	-	2.3	7.7	3.8	-	3.0	-	-	-	-
FaD-CP-top(2)_ar6	-	0.7	10.3	49.1	10.6	5.9	2.2	10.9	-	2.4	0.7	1.8	4.1	1.3

[1]Compositions normalized to 100%

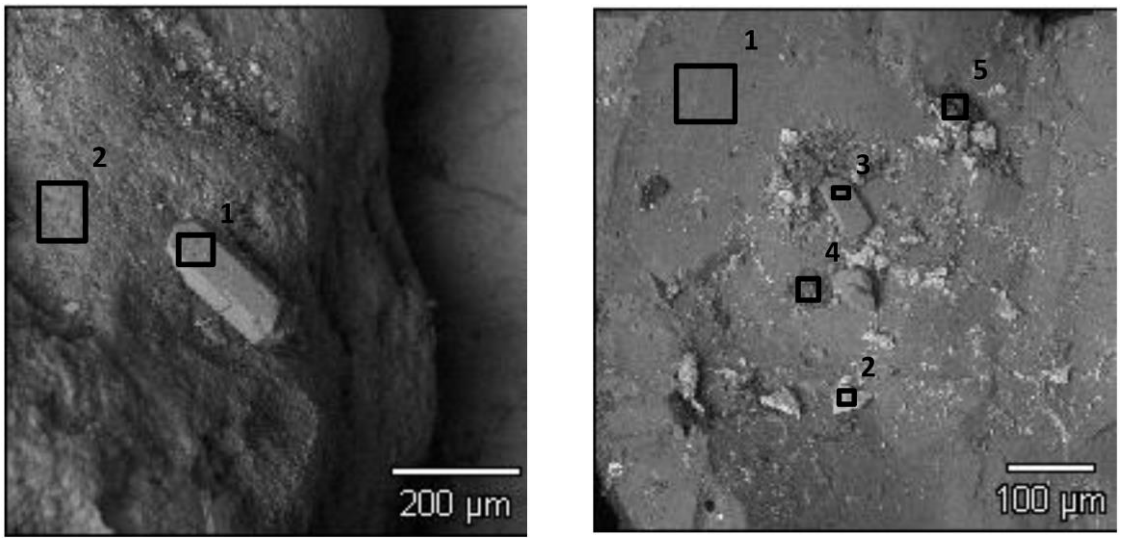
The FaD-CP-top(1) sample contained small particles of Fe (areas 1, 2 and 5) bound with various elements disallowing determination of the form. It can be only presumed that Fe was in a form of(oxy)hydroxides or phosphates. The increased amount of Fe could be attributed to the

oxidizing effect of the plants. The FaD-CP-top(2) had similar composition but small particles were aggregated in a larger cluster containing organic material.

In all the four samples of the substrate from the FaD-CP column no reduced forms of metals were observed. In fact the metals of concern Ni, Cu and Zn, save Al, were not detected.

7.8.2 SEM-EDS analysis of the substrate from the FaD-SP column (system A)

The SEM-BSE images of the samples taken from the bottom part of the FaD-SP column of system A are presented in Fig. 7.22 and the elemental composition of the selected areas are listed in Tab. 7.24



(a) Sample FaD-SP-bot(1) (b) Sample FaD-SP-bot(2)

Fig. 7.22 SEM-BSE images of wetland substrates sampled from the bottom (bot) of the FaD-SP column fed with the cyanide wastewater (system A)

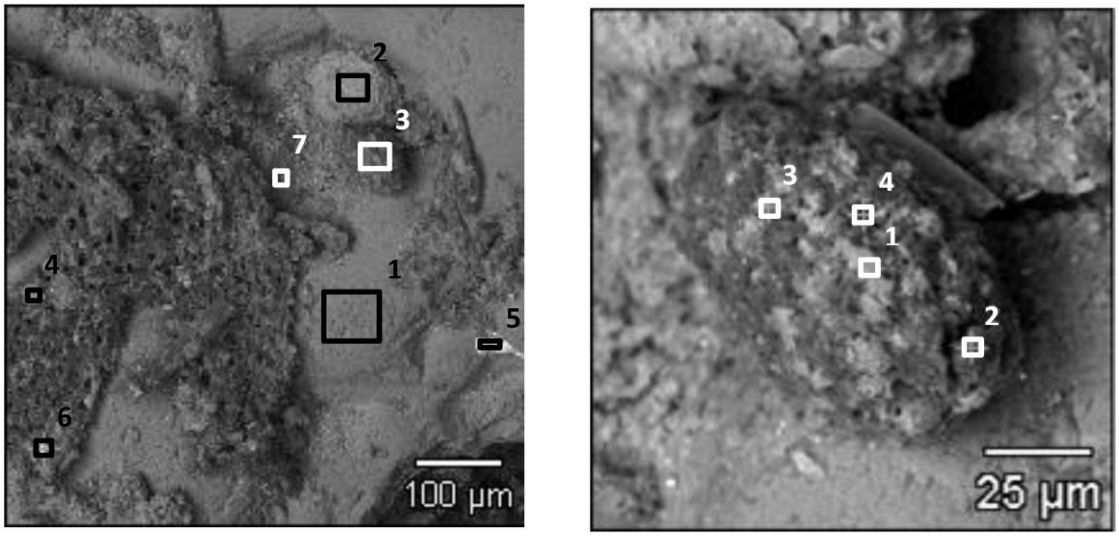
Tab. 7.24 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.22 determined using EDS

Sample_Area	Atomic %[1]									
	Na	**Al**	**Si**	**P**	**S**	**Cl**	**K**	**Ca**	**Ti**	**Fe**
FaD-SP-bot(1)_ar1	1.3	2.8	36.0	-	29.8	-	0.4	26.6	1.1	2.0
FaD-SP-bot(1)_ar2	1.6	6.0	85.7	-	1.8	-	0.5	0.6	1.4	2.5
FaD-SP-bot(2)_ar1	-	0.7	97.5	-	-	-	-	-	1.8	-
FaD-SP-bot(2)_ar2	1.5	4.2	52.3	1.1	2.3	-	-	0.3	1.5	36.8
FaD-SP-bot(2)_ar3	0.4	1.2	35.5	-	30.8	-	0.2	27.5	1.9	2.5
FaD-SP-bot(2)_ar4	1.6	7.5	78.8	-	3.1	-	0.8	0.5	1.9	5.9
FaD-SP-bot(2)_ar5	2.0	5.4	65.6	1.8	3.6	0.4	1.1	-	2.2	17.8

[1]Compositions normalized to 100%

The composition of the FaD-SP-bot(1) sample indicates the presence of calcium sulphate (area 1) and titanium (Ti) as a native component of the medium. The FaD-SP-bot(2) sample contained certain forms of Fe, probably hydroxides or associated with calcium sulphate.

The SEM-BSE images of the samples taken from the top part of the FaD-SP column of system A are presented in Fig. 7.23 and the elemental composition of the selected areas are listed in Tab. 7.25.



(a) Sample FaD-SP-top(1) (b) Sample FaD-SP-top(2)

Figure 7.23 SEM-BSE images of wetland substrates sampled from the top of the FaD-SP column fed with the cyanide wastewater (system A)

Table 7.25 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.23 determined using EDS

Sample_Area	Atomic %[1]														
	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Fe	Ni	Cu	Zr	Sn
FaD-SP-top(1)_ar1	-	-	1.7	95.9	1.5	-	-		0.9	-	-	-		-	-
FaD-SP-top(1)_ar2	1.4	0.9	18.0	57.5	2.0	1.7	-	4.0	1.6	1.2	11.0	-	0.7	-	-
FaD-SP-top(1)_ar3	5.5	1.6	10.2	52.1	4.1	9.6	-	3.3	7.2		2.9	-	3.6	-	-
FaD-SP-top(1)_ar4	0.9	0.2	2.2	22.1	1.3	36.3	-	0.4	34.5	0.3	0.9	-	0.8	-	-
FaD-SP-top(1)_ar5	1.3	-	3.5	75.6	-	2.7	-	1.2	2.8	0.6	3.6	-	-	8.8	-
FaD-SP-top(1)_ar6	5.0	1.3	7.0	41.5	4.6	3.0	-	-	-	-	2.5	1.1	3.3	-	30.7
FaD-SP-top(1)_ar7	1.2	0.5	4.0	86.3	3.4	-	-	0.4	1.3	-	1.5	0.7	0.6	-	-
FaD-SP-top(2)_ar1	2.0	0.8	22.8	53.4	1.9	5.7	-	6.2	4.4	-	1.7	-	1.0	-	-
FaD-SP-top(2)_ar2	8.0	2.1	8.5	52.2	3.1	7.2	0.8	2.7	6.0	0.3	2.6	1.3	5.3	-	-
FaD-SP-top(2)_ar3	6.0	1.5	12.1	36.1	12.9	9.7	-	3.1	8.3	0.4	3.6	0.8	3.4	-	2.1
FaD-SP-top(2)_ar4	8.7	2.1	6.0	54.8	2.3	8.2	0.6	2.4	6.0	-	2.0	1.1	5.6	-	-

[1]Compositions normalized to 100%

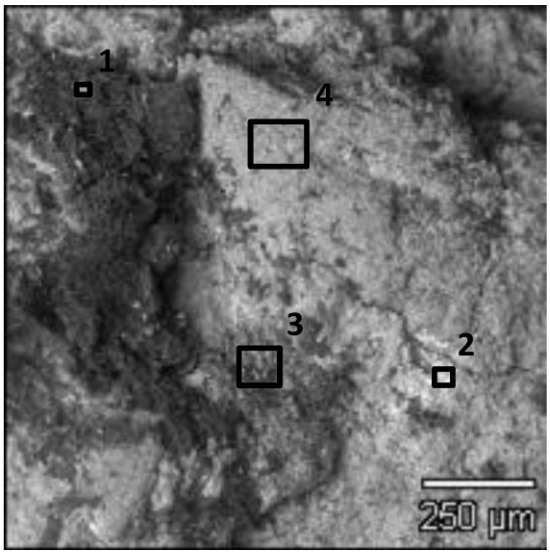
Both samples of the substrate taken from the top part of the FaD-SP column shown in Fig. 7.23 differ from the sample representing the bottom part of the column in that they contained plant detrital matter and the metals of concern (Ni and Cu). These metals were present in small

particles containing Fe and sulphur, which may suggest the presence of sulphides, but also the presence of oxidized forms of metals cannot be excluded. Sample FaD-SP-top(1) contained calcium sulphate (area 4) and probably elemental tin (area 6).

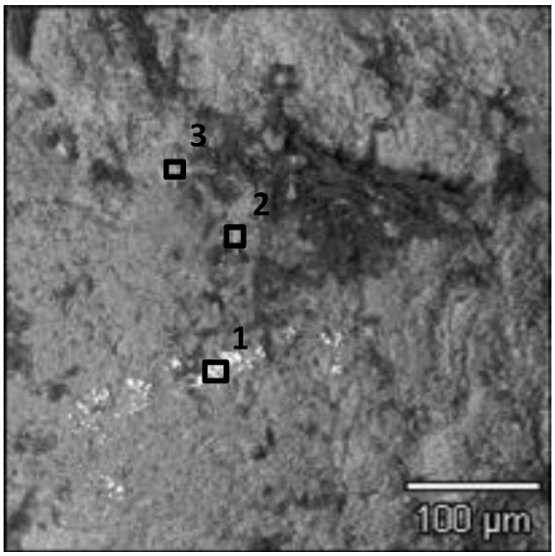
These finding are in agreement with the chemical analysis of the substrate from the FaD-SP column. As it was shown in Fig. 7.14 and Tab. 7.20 the concentration of Cu and Ni was markedly higher in the top part of the column and was mainly present in the exchangeable and reducible fraction.

7.8.3 SEM-EDS analysis of the substrate from the UF-PP column (system B)

The SEM-BSE images of the samples taken from the bottom part of the UF-PP column of system B are presented in Fig. 7.24 and the elemental composition of the selected areas are listed in Tab. 7.26.



(a) Sample UF-PP-bot(1)



(b) Sample UF-PP-bot(2)

Figure 7.24 SEM-BSE images of wetland substrates sampled from the bottom (bot) of the UF-PP column (system B)

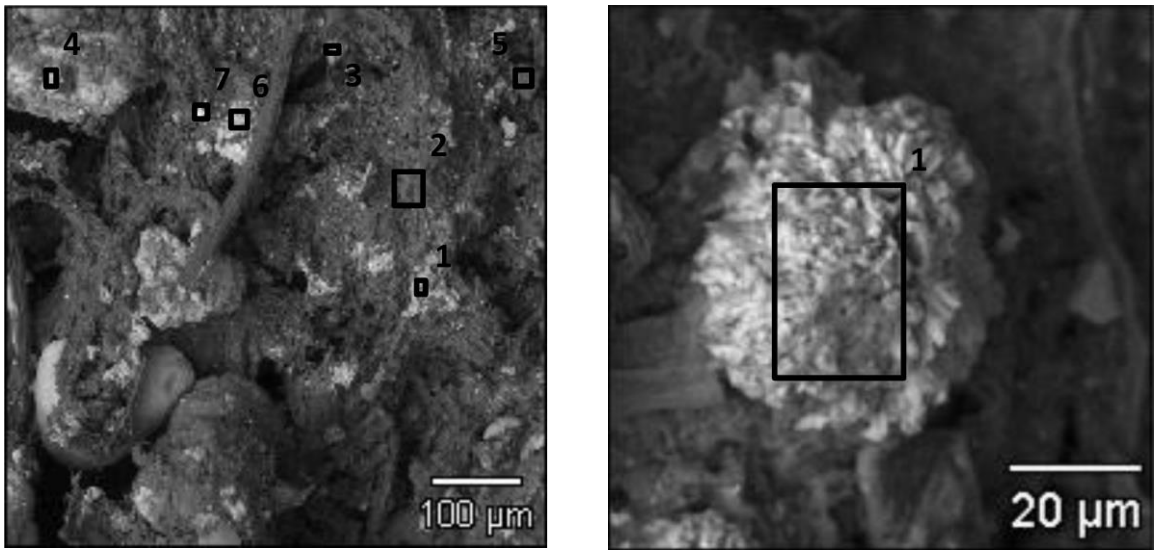
Table 7.26 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.24 determined using EDS

Sample_Area	Atomic %[1]										
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Ti**	**Fe**	**Ni**
UF-PP-bot(1)_ar1	2.9	2.5	16.2	56.7	-	2.7	10.2	3.4		5.3	-
UF-PP-bot(1)_ar2	3.4	1.2	14.1	59.1	-	1.5	3.8	1.0	0.6	15.3	-
UF-PP-bot(1)_ar3	2.4	1.7	16.6	64.2	-	2.4	7.5	2.4	-	2.7	-
UF-PP-bot(1)_ar4	8.6	0.8	16.6	69.7	-	-	2.2	0.9	-	1.2	-
UF-PP-bot(2)_ar1	-	-	3.9	78.8	-	-	1.2	0.5	-	-	15.6
UF-PP-bot(2)_ar2	1.0	4.5	13.9	60.7	-	0.7	7.5	-	1.7	9.8	-
UF-PP-bot(2)_ar3	1.1	0.6	5.6	30.9	23.0	0.5	2.2	35.5	-	0.6	-

[1]Compositions normalized to 100%

It can be seen that the grain of gravel had some peat attached to it, in which small bright particles were present, indicating their higher mean atom mass resulting probably from the presence of Fe. The amount of S is small suggesting that sulphides were not abundant in the top part of the UF-PP column. The UF-PP-bot(2) sample contained elevated amount of Ni but it was probably of native origin.

The SEM-BSE images of the samples taken from the top part of the UF-PP column of system B are presented in Fig. 7.25 and the elemental composition of the selected areas are listed in Tab. 7.27.



(a) Sample UF-PP-top(1) (b) Sample UF-PP-top(2)

Figure 7.25 SEM-BSE images of wetland substrates sampled from the top of the UF-PP column (system B)

Table 7.27 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.25 determined using EDS

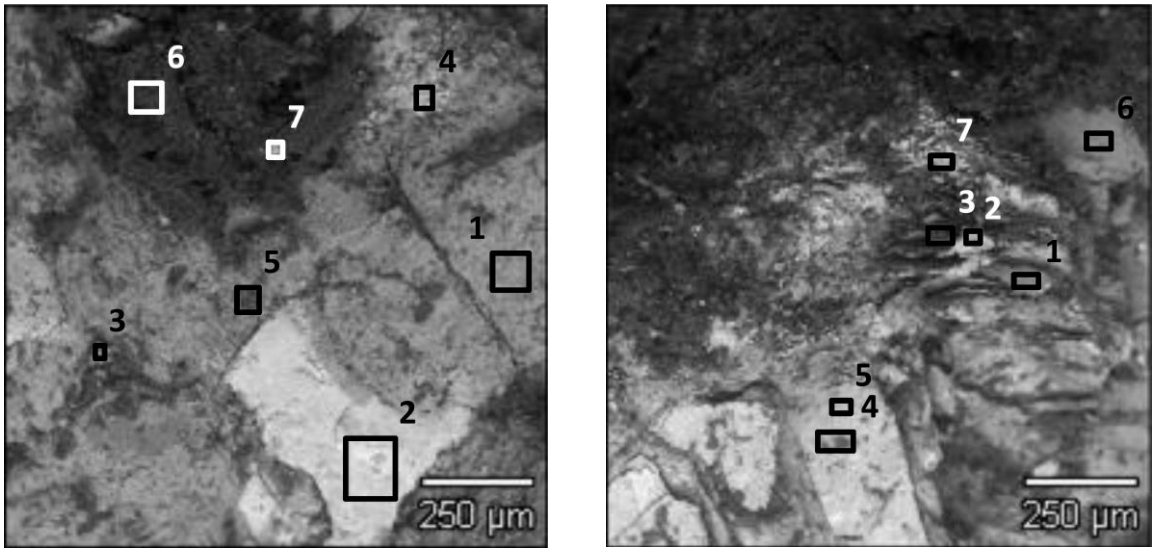
Sample_Area	Atomic %[1]										
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**Cl**	**K**	**Ca**	**Mn**	**Fe**
UF-PP-top(1)_ar1	1.0	0.6	1.1	4.8	-	43.0		0.4	48.2	-	0.9
UF-PP-top(1)_ar2	8.6	5.4	5.9	24.4	1.1	20.6	2.7	2.8	26.0	-	2.6
UF-PP-top(1)_ar3	1.2	0.7	1.0	3.5	-	45.3	-	-	48.3	-	-
UF-PP-top(1)_ar4	1.6	0.7	1.3	9.2	-	41.6	-	-	44.6	-	1.0
UF-PP-top(1)_ar5	9.3	7.1	4.8	14.9	-	22.5	5.1	-	32.9	-	3.5
UF-PP-top(1)_ar6	1.1	0.7	1.2	4.6	-	45.9	-	0.4	46.1	-	-
UF-PP-top(1)_ar7	2.7	1.5	2.0	7.1	0.3	52.2	-	0.4	6.7	0.6	26.5
UF-PP-top(2)_ar1	1.2	0.5	0.6	3.3	0.3	45.9	-	-	47.5	-	0.6

[1]Compositions normalized to 100%

Both UF-PP-top samples contain the amounts of Ca and S suggesting the presence of gypsum ($CaSO_4 \cdot 2H_2O$). The increased Fe concentration was observed in the area 7 of the UF-PP-top(1) sample. The characteristics of the adjacent areas may suggest that Fe is not present in sulphide form, but rather as oxidized species, namely (oxy)hydroxides.

7.8.4 SEM-EDS analysis of the substrate from the UF-GP column (system B)

The SEM-BSE images of the samples taken from the bottom part of the UF-GP column of system B are presented in Fig. 7.26 and the elemental composition of the selected areas are listed in Tab. 7.28.



(a) Sample UF-GP-bot(1) (b) Sample UF-GP-bot(2)

Figure 7.26 SEM-BSE images of wetland substrates sampled from the bottom (bot) of the UF-GP column (system B)

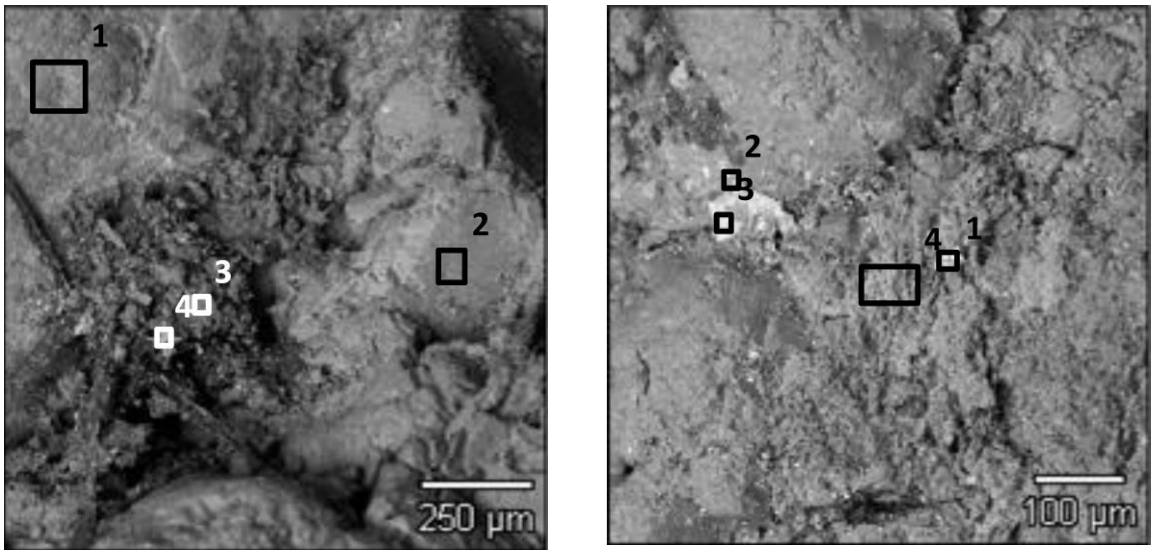
Table 7.28 Elemental (atomic %) composition of the areas (ar#) indicted in the images in Fig. 7.26 determined using EDS

Sample_Area	Atomic %[1]										
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Ti**	**Mn**	**Fe**
UF-GP-bot(1)_ar1	7.2	0.6	24.7	59.0	-	0.9	3.3	1.9	-	-	2.4
UF-GP-bot(1)_ar2	1.9	7.3	20.7	35.1	-	6.1	2.0	1.1	1.9	-	23.9
UF-GP-bot(1)_ar3	3.8	1.0	15.1	40.9	-	1.3	6.1	5.7	4.6	-	21.6
UF-GP-bot(1)_ar4	3.2	4.1	16.9	35.1	0.5	8.9	2.7	2.4	2.9	-	23.3
UF-GP-bot(1)_ar5	6.7	1.3	23.2	54.0	-	2.4	3.2	4.4	-	-	4.9
UF-GP-bot(1)_ar6	6.1	2.5	13.9	36.1	1.1	9.6	2.3	16.2	0.9	-	11.3
UF-GP-bot(1)_ar7	3.9	1.3	8.0	20.4	0.6	26.9	0.9	5.2	-	0.5	32.1
UF-GP-bot(2)_ar1	0.8	10.1	19.8	29.7	-	4.1	1.0	1.2	0.8	0.8	31.8
UF-GP-bot(2)_ar2	1.0	6.3	14.8	26.4	-	4.2	4.9	1.3	2.6	0.7	37.9
UF-GP-bot(2)_ar3	1.3	7.3	17.4	29.8	-	5.8	3.6	1.7	2.8	0.5	29.7
UF-GP-bot(2)_ar4	1.2	7.7	16.9	26.8	0.4	4.6	2.2	1.9	1.3	0.7	36.3
UF-GP-bot(2)_ar5	0.7	6.4	16.1	26.9	-	2.9	4.2	1.2	3.1	0.5	37.9
UF-GP-bot(2)_ar6	-	0.7	9.6	79.0	-	1.4	3.4	1.3	-	-	4.6
UF-GP-bot(2)_ar7	0.9	7.1	15.7	28.7	-	9.1	2.5	1.8	1.5	-	32.7

[1]Compositions normalized to 100%

Both samples had similar composition characterized by elevated amounts of Fe accompanied by much smaller amounts of Mn. It was also observed that the increased concentration of Fe corresponded to elevated amounts of S, which may indicate the presence of sulphides (e.g. area 7 of sample UF-GP-bot(1)). This may also suggest that Fe^{II} competes for sulphide with other cations, which were present in the feed as was observed by Song (2003). Some of the areas observed on the analysed material seemed to be native to the substrate, e.g. area 2 of sample UF-GP-bot(1), and areas 4-5 of sample UF-GP-bot(2).

The SEM-BSE images of the samples taken from the top part of the UF-GP column of system B are presented in Fig. 7.27 and the elemental composition of the selected areas are listed in Tab. 7.29.



(a) Sample UF-GP-top(1) (b) Sample UF-GP-top(2)

Figure 7.27 SEM-BSE images of wetland substrates sampled from the top of the UF-GP column (system B)

Table 7.29 Elemental (atomic %) composition of the areas (ar#) indicted in the images in Fig. 7.27 determined using EDS

Sample_Area	Atomic %[1]														
	Na	Mg	Al	Si	P	S	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn
UF-GP-top(1)_ar1	8.4		19.1	61.3	-	2.5	3.3	2.1	-	-	-	3.3	-	-	-
UF-GP-top(1)_ar2	1.9	1.0	28.7	52.2	0.7	1.1	11.4	-	0.2	-	-	2.8	-	-	-
UF-GP-top(1)_ar3	3.4	0.4	5.8	82.1	0.8	1.7	1.5	1.2	-	-	-	2.1	-	-	-
UF-GP-top(1)_ar4	4.2	5.6	17.2	47.5	0.6	2.2	5.0	1.6	1.3	-	-	13.2	0.8	-	-
UF-GP-top(2)_ar1	3.0	0.7	8.8	23.1	-	0.6	1.9	0.7	-	10.9	0.8	44.5	5.1	-	-
UF-GP-top(2)_ar2	-	0.8	9.6	45.2	1.5	14.3	1.8	2.1	-	-	-	6.8	-	0.8	17.1
UF-GP-top(2)_ar3	2.5	3.7	16.5	51.1	0.7	1.1	7.3	1.1	1.2	-	-	14.9	-	-	-
UF-GP-top(2)_ar4	2.4	1.5	26.1	53.1	0.7	0.9	10.9	-	0.2	-	-	4.2	-	-	-

[1]Compositions normalized to 100%

The EDS analysis of the sample from the top part of the UF-GP column indicated the presence of the metals of concern: Ni, Cu and Zn. Area 2 of sample UF-GP-top(2) contains elevated amounts of S and Zn, which may suggest the presence of sulphides. Also area 4 of the UF-GP-top(2) may contain sulphides, however, the amount of S is small. The composition of area 1 of sample UF-GP-top(2) is untypical as it contains chromium (Cr), which was not added to the feed and it is likely that it was native to the substrate.

7.8.5 SEM-EDS analysis of the substrate from the FaD-PP column (system B)

All the previously presented SEM-BSE images and the results of the EDS analyses indicated that the metals of concern were not abundant in the substrate and in most cases were not detected. The visual appearance of the gravel or sand sampled from the columns of system A or B did not

indicate clearly the presence of metal sulphides. In fact, these naked-eye observations were confirmed to some extent by the SEM-EDS analyses. The observed particles assumed to be sulphides were small and unevenly distributed on the substrate surface and were not forming larger clusters. In contrast to the above-mentioned samples the typical appearance of sulphide tarnish was observed on the gravel found on the very bottom and in the outlet stub of the FaD-PP column of system B. The SEM-BSE image of the surface of this sample is presented in Fig. 7.28.

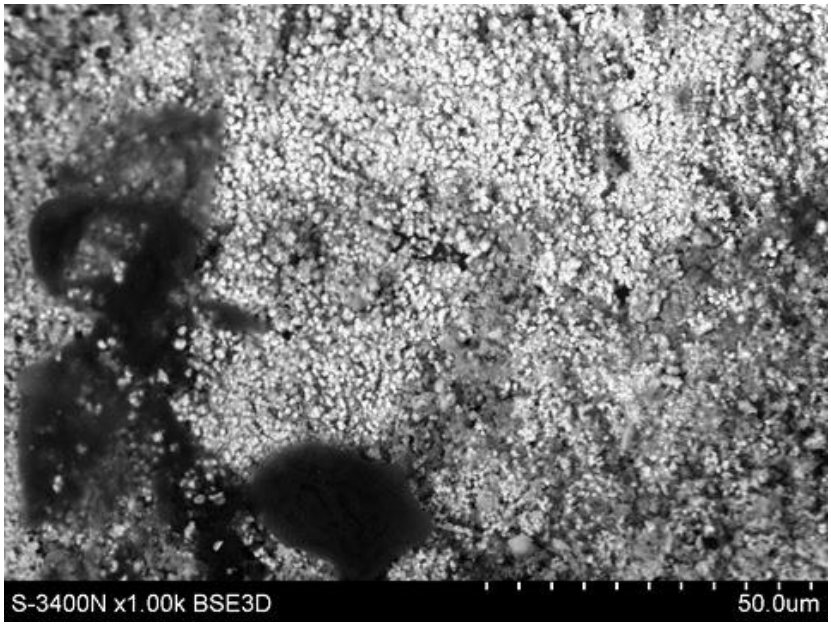


Figure 7.28 SEM-BSE image of a gravel grain collected in the outlet of the FaD-PP column

The grainy clusters observed on the surface of gravel had the appearance of the sulphide framboids. The composition mapping was performed to corroborate this hypothesis (Fig. 7.29).

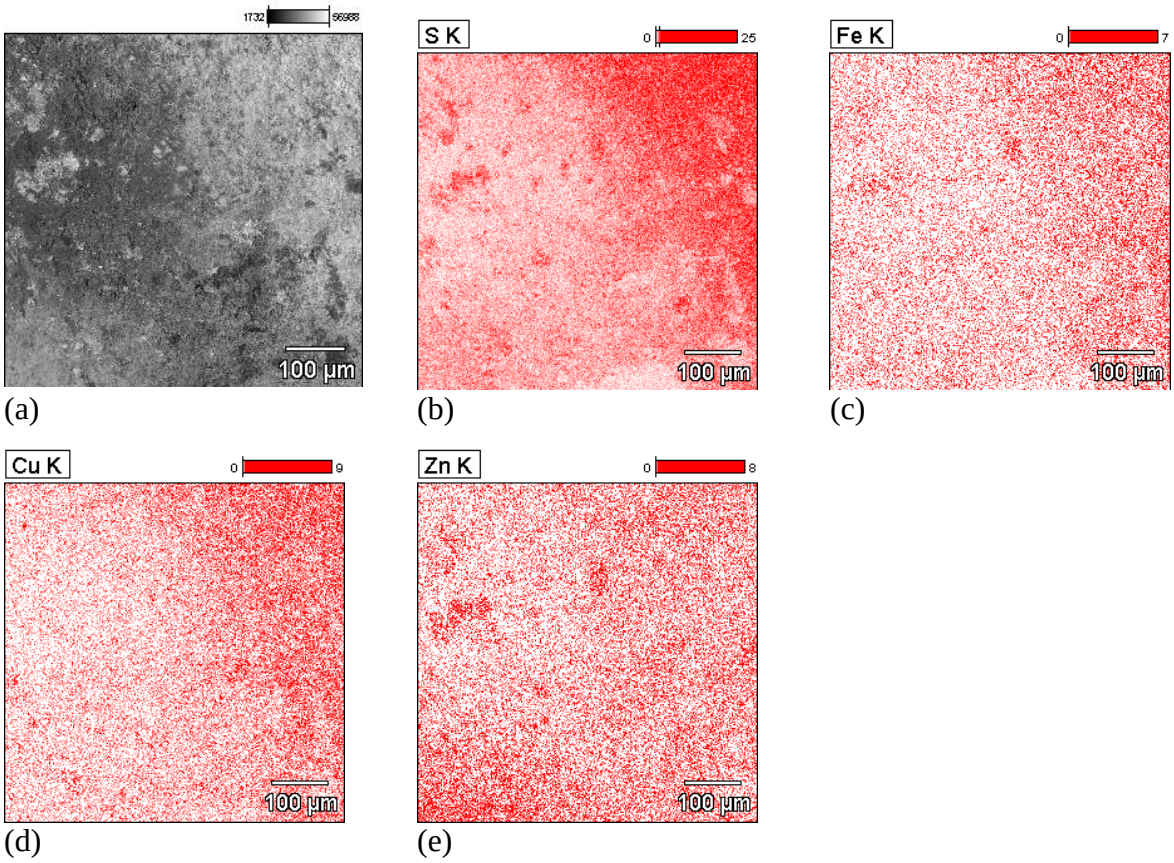
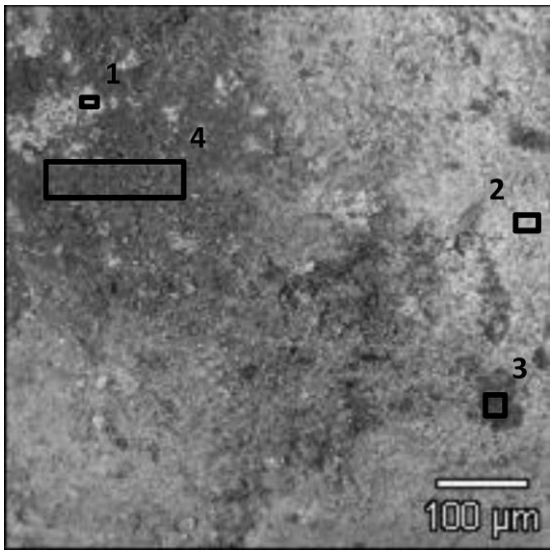
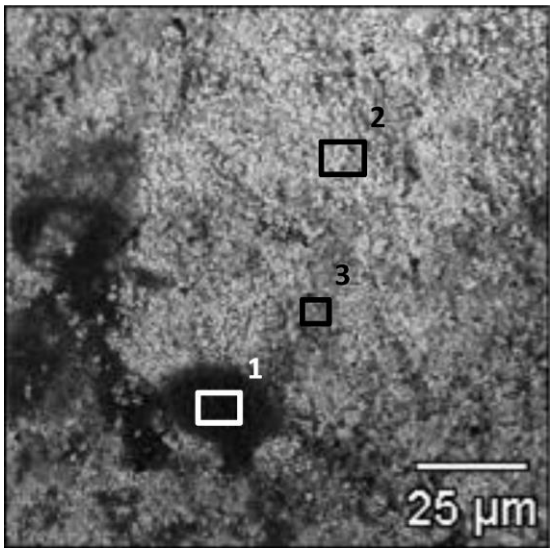


Figure 7.29 Composition mapping of sulphur (b) iron (c), copper (d) and zinc (e) showing their presence on a surface of the material (red dots). Image (a) shows general SEM-BSE image of the selected region with the brighter hue for the higher mean atomic mass spots.

It can be seen that S is mostly accompanied by Fe and Cu in the upper, left-hand corner of the image, which indicates the presence of Cu and Fe sulphides. It was also observed that the distribution pattern for Zn was different than for Cu and Fe, but the presence of the Zn sulphides should not be excluded. The results of the EDS analyses of the sample presented in Fig. 7.29 (with the analysed areas in Fig. 7.30) and another sample (image (b) in Fig. 7.30) are given in Tab. 7.30.



(a) Sample FaD-PP-out(1)



(b) Sample FaD-PP-out(2)

Figure 7.30 SEM-bSE images of wetland substrates sampled from outlet pipe (out) of the FaD-PP column (system B)

Table 7.30 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.30 determined using EDS

Sample_Area	**Atomic %**[1]									
	Al	**Si**	**P**	**S**	**Cl**	**K**	**Ca**	**Fe**	**Cu**	**Zn**
FaD-PP-out(1)_ar1	7.9	21.3	-	29.2	2.4	0.4	1.8	4.3	7.1	25.6
FaD-PP-out(1)_ar2	2.7	8.1	0.2	38.8	1.4	-	1.6	5.7	28.0	13.4
FaD-PP-out(1)_ar3	16.1	37.4	-	16.4	0.8	1.2	2.4	3.1	11.5	11.3
FaD-PP-out(1)_ar4	16.2	42.6	-	14.8	1.5	1.1	2.9	2.2	8.9	9.8
FaD-PP-out(2)_ar1	1.0	4.3	-	42.8	8.7	4.2	2.2	4.9	22.6	9.3
FaD-PP-out(2)_ar2	-	2.0	-	43.8	1.0	-	0.9	6.3	40.3	5.7
FaD-PP-out(2)_ar3	0.5	3.8	-	41.5	1.7	-	1.0	6.7	33.5	11.4

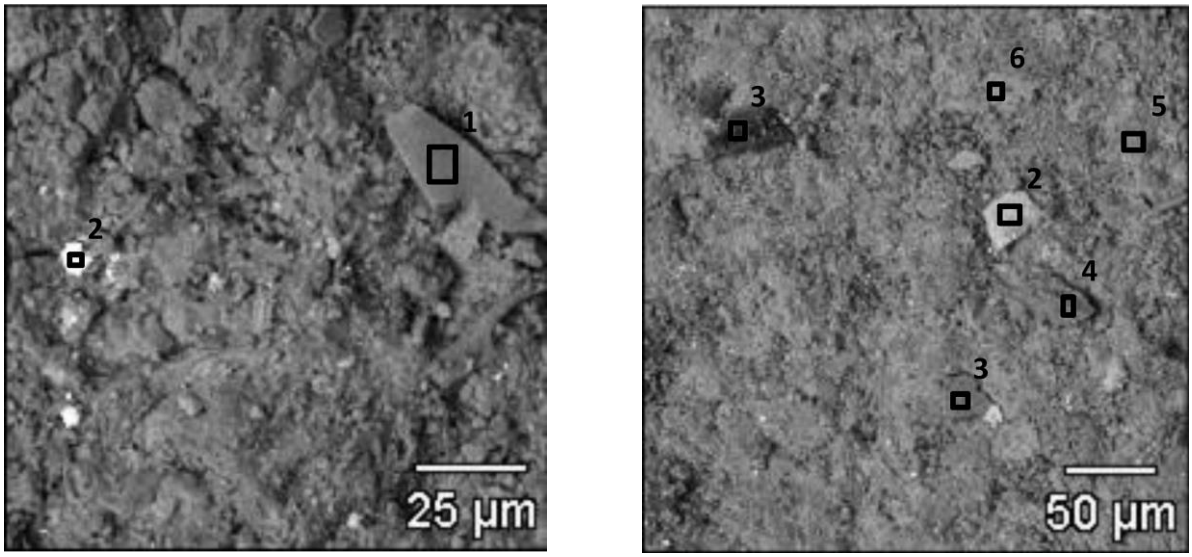
[1]Compositions normalized to 100%

The EDS analysis of the sample shown in Fig. 7.29 and 7.30 (image (a)) indicates that high amounts of S were accompanied by Cu and Zn. The analysed area 2 is within the region of the sample (FaD-PP-out(1)) for which the elemental mapping was performed (Fig. 7.29). The EDS analysis for this area yields composition that corroborates the previously suggested hypothesis. In fact, this area contains mostly Cu and sulphur but Zn is also present in concentration higher than for Fe. Interestingly, Zn is also present in a separate cluster indicated as area 1 in the image presenting sample FaD-PP-out(1) (Fig. 7.30). This cluster contains mostly Zn (25.6%) and sulphur (29.2%) and markedly lower amount of Cu (7.1%), which can be also noticed in Fig. 7.29 where several Zn-S clusters are visible. Also the other areas of sample FaD-PP-out(1) contain significant amounts of S, Cu and Zn, however, smaller than in areas 1 and 2. The sample with the sulphide framboids (Fig. 7.28) was also analysed by the EDS with the selected areas shown in Fig. 7.30 (image (b), FaD-PP-out(2)). The selected area of sample FaD-PP-out(2)

contained even more Cu and S than in the previously analysed sample. As it was observed the concentration of Cu was higher than Zn in all the areas by 2- to 8-fold. In both samples Fe accompanied the other metals and S and was probably also bound in the form of sulphide.

7.8.6 SEM-EDS analysis of the substrate from the FaD-GP column (system B)

The SEM-BSE images of the samples taken from the bottom part of the FaD-GP column of system B are presented in Fig. 7.31 and the elemental composition of the selected areas are listed in Tab. 7.31.



(a) Sample FaD-GP-bot(1) (b) Sample FaD-GP-bot(2)

Figure 7.31 SEM-BSE images of wetland substrates sampled from the bottom (bot) of the FaD-GP column (system B)

Table 7.31 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.31 determined using EDS

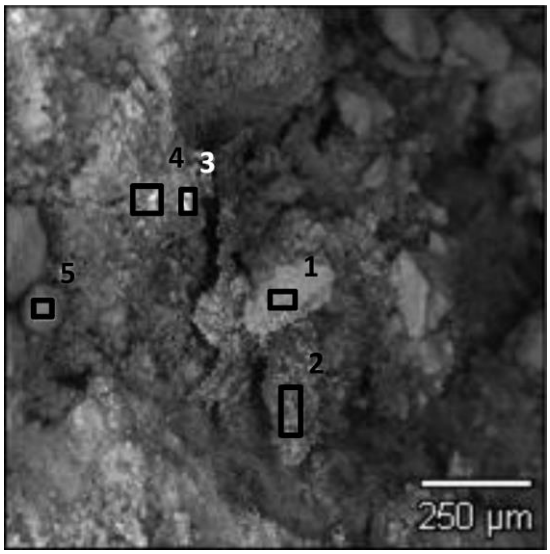
Sample_Area	Atomic %[1]										
	Na	**Mg**	**Al**	**Si**	**S**	**K**	**Ca**	**Ti**	**Fe**	**Zr**	**Ag**
FaD-GP-bot(1)_ar1	1.9	0.9	20.6	64.8	-	9.7	-	-	2.1	-	-
FaD-GP-bot(1)_ar2	3.5	1.0	10.9	53.5	-	3.9	3.4	-	3.1	19.4	1.2
FaD-GP-bot(2)_ar1	1.5	0.4	6.1	86.7	-	3.6	0.8	-	0.9	-	-
FaD-GP-bot(2)_ar2	2.6	4.5	16.6	53.4	0.4	7.5	-	1.3	13.7	-	-
FaD-GP-bot(2)_ar3	3.4	0.5	11.4	73.3	-	4.8	3.4	1.5	1.7	-	-
FaD-GP-bot(2)_ar4	1.2	-	6.0	88.5	-	3.2	-	-	1.1	-	-
FaD-GP-bot(2)_ar5	2.0	0.5	6.7	86.0	-	3.6	-	-	1.2	-	-
FaD-GP-bot(2)_ar6	2.5	-	16.2	65.9	-	14.1	-	-	1.3	-	-

[1]Compositions normalized to 100%

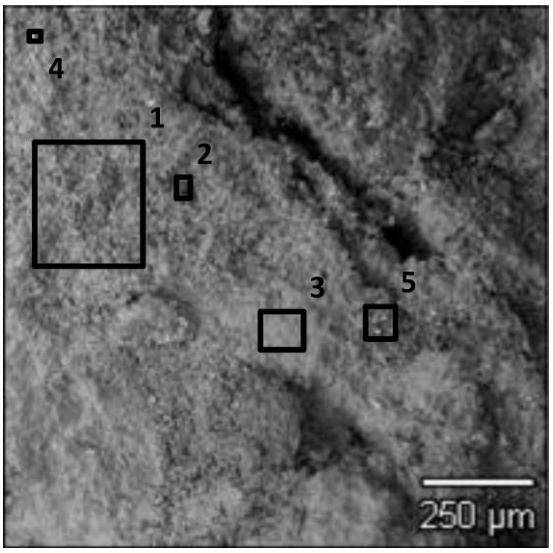
The EDS analysis of the samples collected from the bottom part of the FaD-GP column shows that S was not present in detectable concentrations. Also none of the metals of concern were

detected. It can be assumed that the composition of the samples was similar to the raw gravel (Tab. 7.34). The detected amounts of zirconium (Zr), titanium (Ti) and silver (Ag) were related to the native components of gravel.

The SEM-BSE images of the samples taken from the top part of the FaD-GP column of system B are presented in Fig. 7.32 and the elemental composition of the selected areas are listed in Tab. 7.32.



(a) Sample FaD-GP-top(1)



(b) Sample FaD-GP-top(2)

Figure 7.32 SEM-BSE images of wetland substrates sampled from the top of the FaD-GP column (system B)

Table 7.32 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.32 determined using EDS

Sample_Area	Atomic %[1]													
	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Fe	Ni	Cu	Zn
FaD-GP-top(1)_ar1	-	8.8	23.2	43.4	-	0.9	-	7.1	1.5	1.7	10.6	1.1	0.9	0.8
FaD-GP-top(1)_ar2	6.4	2.4	14.8	49.6	1.2	4.4	0.5	2.8	10.1	0.4	3.8	1.2	2.5	-
FaD-GP-top(1)_ar3	6.5	1.3	9.8	43.4	0.4	2.4	-	1.1	3.7	-	29.7	0.7	1.0	-
FaD-GP-top(1)_ar4	9.6	2.4	9.4	47.5	0.7	3.0	-	1.3	5.2	0.9	15.1	2.5	2.5	-
FaD-GP-top(1)_ar5	0.7	0.6	4.6	87.8	-	1.5	-	1.1	1.5	-	2.2	-	-	-
FaD-GP-top(2)_ar1	2.1	0.9	23.7	51.5	0.4	3.6	-	6.8	4.4	1.9	3.7	1.0	-	-
FaD-GP-top(2)_ar2	1.8	1.8	17.1	46.2	2.5	4.0	0.4	5.6	8.5	1.2	5.1	1.7	2.0	2.1
FaD-GP-top(2)_ar3	1.4	1.2	17.3	60.0	-	4.6	-	6.0	4.3	0.6	4.6	-		-
FaD-GP-top(2)_ar4	3.4	0.7	15.9	35.9	0.2	2.6	-	2.6	3.5	31.0	4.2	-	-	-
FaD-GP-top(2)_ar5	4.7	0.8	23.0	55.3	-	1.6	-	2.6	1.9	0.6	9.6	-	-	-

[1]Compositions normalized to 100%

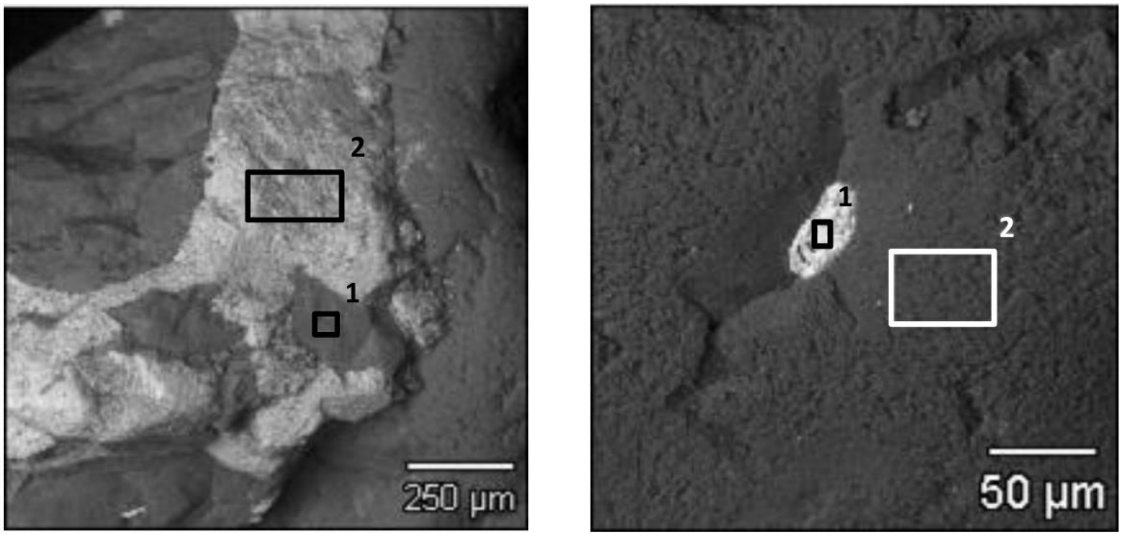
In contrast to the bottom samples the elemental composition of the gravel sampled from the top part of the FaD-GP column contained detectable amounts of S and the metals that were present in the feed. Based on the fact that this column was used to treat the influent containing high concentrations of metals emulating the raw electroplating wastewater (Section 7.4) it was

expected that increased amounts of contaminants could be present in top layer of substrate of the column. Although S was present it is suggested that it was rather sulphate S than sulphide S. It is also likely that the metals were present in the oxidized form (hydroxides) rather than reduced (sulphides). Interestingly, these findings are similar to those for the counterpart of the FaD-GP columns in system A, namely the FaD-SP column.

7.8.7 SEM-EDS analysis of the raw gravel and sand

The above-presented results of the SEM-EDS analysis of the spent substrate contained several areas in which the elemental composition was much different from the other analysed areas. In this situation it was assumed that the detected elements were of native origin. This means that they were present in the media before filling the columns. There was certain evidence, however indirect, that the bed media contained some contaminants that could have been leached into the wastewater flowing through the columns. This was especially a case for Mn and Fe. The chemical analysis confirmed these assumptions for sand and compost used in system A. The SEM-EDS analysis was performed to furnish more information on the forms of native contaminants in the raw media.

The SEM-BSE images of the samples of sand used in system A are presented in Fig. 7.33 and the elemental composition of the selected areas are listed in Tab. 7.33.



(a) Sample Sand(1) (b) Sample Sand(2)

Figure 7.33 SEM-BSE images of the raw sand used as a bed medium in system A

Table 7.33 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.33 determined using EDS

Sample_Area	Atomic %[1]					
	Al	**Si**	**Ca**	**Ti**	**Fe**	**Zr**
Sand(1)_ar1	1.9	88.0	-	0.6	9.5	-
Sand(1)_ar2	4.2	29.6	0.8	0.5	64.7	0.3
Sand(2)_ar1	2.0	68.9	1.8	-	-	27.3
Sand(2)_ar2	2.6	96.8	0.6	-	-	-

[1]Compositions normalized to 100%

The sand used in system A contained invariably silica (Si) and much lower amounts of Al. The other elements that were detected were Ca, Ti, Fe and Zr. Iron was present in the form of oxide and could have been leached from the sand in anaerobic conditions.

The SEM-BSE images of the samples of the raw gravel used in system B are presented in Fig. 7.34 and the elemental composition of the selected areas are listed in Tab. 7.34.

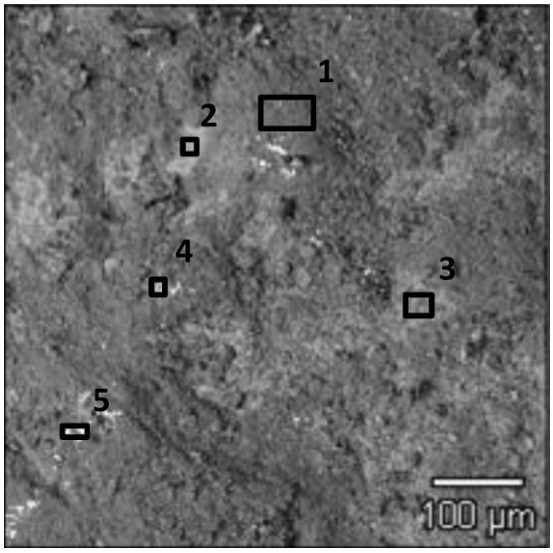


Figure 7.34 SEM-BSE image of the raw gravel used as a bed medium in system B: Gravel(1)

Table 7.34 Elemental (atomic %) composition of the areas (ar#) indicated in the image in Fig. 7.34 determined using EDS

Sample_Area	Atomic %[1]									
	Na	**Mg**	**Al**	**Si**	**K**	**Ca**	**Ti**	**Fe**	**Co**	**Ni**
Gravel(1)_ar1	0.6	0.6	10.3	82.8	2.0	0.7	-	3.1	-	-
Gravel(1)_ar2	0.6	5.3	20.5	51.9	8.5	-	1.4	11.7	-	-
Gravel(1)_ar3	0.9	3.7	26.5	53.1	6.3	-	1.0	8.6	-	-
Gravel(1)_ar4	0.9	1.0	11.6	34.5	1.4	-	0.2	2.3	0.9	47.2
Gravel(1)_ar5	1.3	1.5	14.0	66.6	1.5	1.1	0.3	3.2	-	10.4

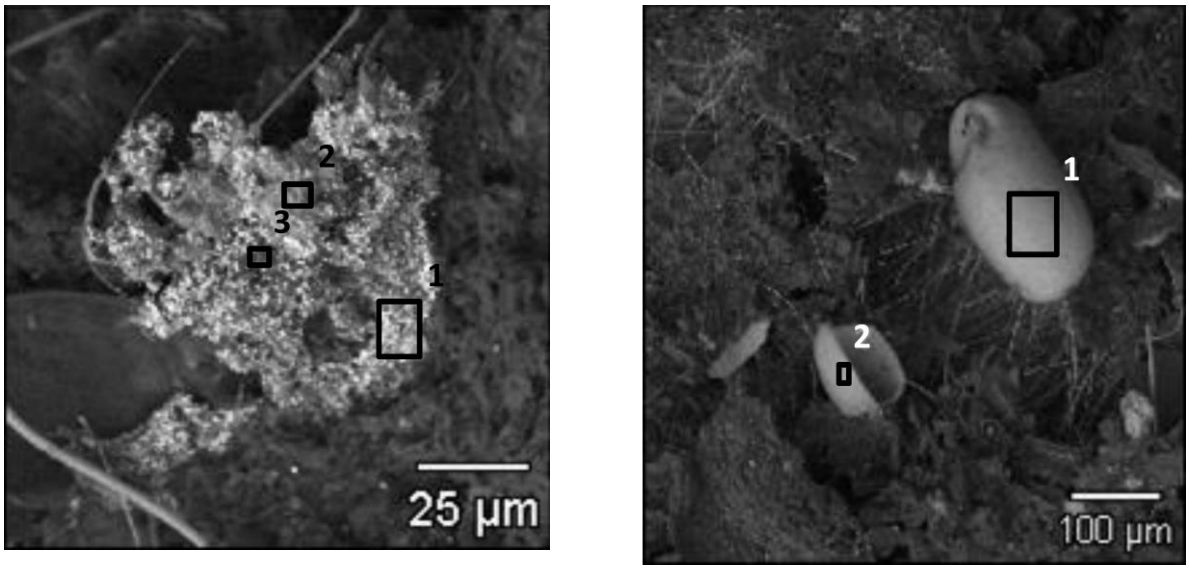
[1]Compositions normalized to 100%

It can be observed that the surface of the gravel is rougher comparing to the sand (Fig. 7.33). This property of the material could be essential as it provides better substrate for bacterial

attachment and biofilm formation. The elemental composition indicates that the gravel contained more impurities that could have been leached into the wastewater. Noteworthy, Fe was found in all the analysed areas, Ni was found in two areas, and Co was found once. The sand used in system A was attested filter sand, and the gravel used in system B was an inexpensive material for gardening.

7.8.8 Analysis of suspended solids retained on filters

The concentration of contaminants was only analysed in filtered samples of the influent and effluent in both system A and B. This is a common practice, however, certain metals species (e.g. aggregated hydroxides or sulphides) may not pass through filter, markedly overestimating the removal efficiency of the system. To assess the amount of contaminants retained on a filter 1.5 L of effluents from the UF-PP and UF-GU columns were filtered through the 0.45 µm filter. This analysis is not fully representative as the sediments on the surface of the bed media in the columns were resuspended while sampling the wastewater above the surface of the bed. Resuspension of the sediments was aimed at analysing the white precipitates easily noticeable in the UF-GU column and presumed to be elemental S. These precipitates were not observed in the UF-PP and UF-PU columns, which could be attributed to the entrapment of the precipitates in a thick layer of sediments (originating from the peat) in these columns. The SEM-BSE images of the filter through which the wastewater from the UF-PP column was filtered are presented in Fig. 7.35 and the elemental composition of the selected areas are listed in Tab. 7.35.



(a) Sample UF-PP-filter(1) (b) Sample UF-PP-filter(2)

Figure 7.35 SEM-BSE images of the filter surface through which the effluent from the UF-PP column was passed (system B)

Table 7.35 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.35 determined using EDS

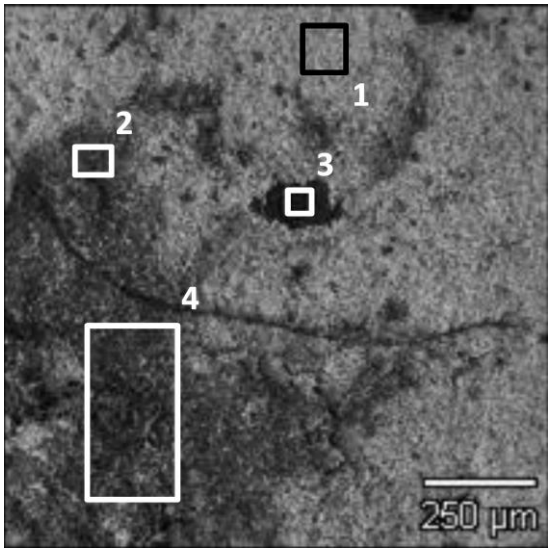
Sample_Area	Atomic %[1]												
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Ti**	**Mn**	**Fe**	**Ni**	**Zn**
UF-PP-filter(1)_ar1	2.2	0.8	1.7	8.1	0.5	18.1	0.6	5.5	0.2	0.8	34.6	17.8	9.1
UF-PP-filter(1)_ar2		0.1	0.7	3.4	0.4	47.1		37.8			5.0	2.1	3.4
UF-PP-filter(1)_ar3	2.1	0.9	2.0	8.5	0.9	44.4	0.7	10.8		0.4	15.4	6.6	7.3
UF-PP-filter(2)_ar1			1.0	5.7		2.4	0.4	83.7			1.3		1.3
UF-PP-filter(2)_ar2	2.4	1.1	2.1	9.4	0.7	3.9	0.6	71.6			1.5		1.5

[1]Compositions normalized to 100%

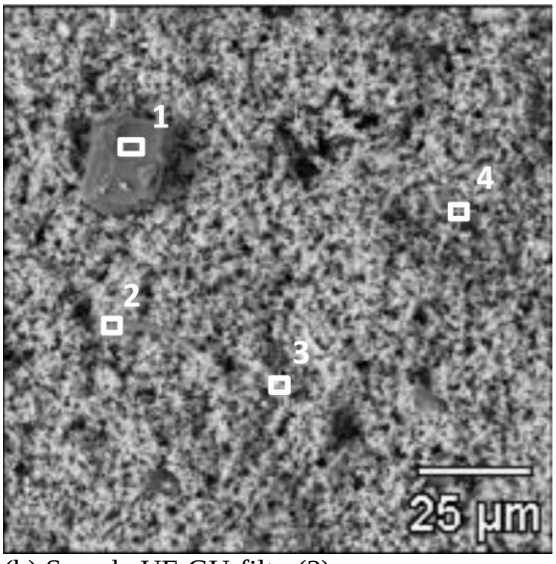
The elemental composition of the solids retained on filter indicates elevated concentration of S and two of the metals of concern: Ni and Zn. The presence of elemental S cannot be excluded, however, the S species present in the sample could have been rather sulphate and sulphide. Sulphate was probably present in area 2 of the UF-PP-filter(1) sample as calcium sulphate. This compound could form when the substrate was dried for the analysis. Interestingly, Cu was not detected in the two areas analysed on the filter, as in the other two samples taken from this column (Section. 7.8.3). The properties of the sediments in the UF-PP columns disallow observation and analysis of small particles such as elemental S or sulphides. The hypothesis on the presence of elemental S cannot be corroborated nor rejected. The egg-shaped objects in Fig. 7.35 (image (b)) were probably cysts of *Daphnia* (observed in this column; Section 7.11.3) or *Oligochaeta*. The lack of peat particles in the UF-GU allowed observation of dilute skim milk appearance in this column, which was caused probably by elemental S (Fig. 7.36). It was hypothesized that the occurrence of elemental S was the effect of sulphur-oxidizing bacteria as *Beggiatoa* that incompletely oxidize sulphide to elemental S (Gammons and Frandsen, 2001).

Figure 7.36 Outflow of the UF-GU column in system B with visible white precipitates

The SEM-BSE images of the filter through which the wastewater from the UF-GU column was filtered are presented in Fig. 7.37 and the elemental composition of the selected areas are listed in Tab. 7.36.



(a) Sample UF-GU-filter(1)



(b) Sample UF-GU-filter(2)

Figure 7.37 SEM-BSE images of the filter surface through which the effluent from the UF-GU column was passed (system B)

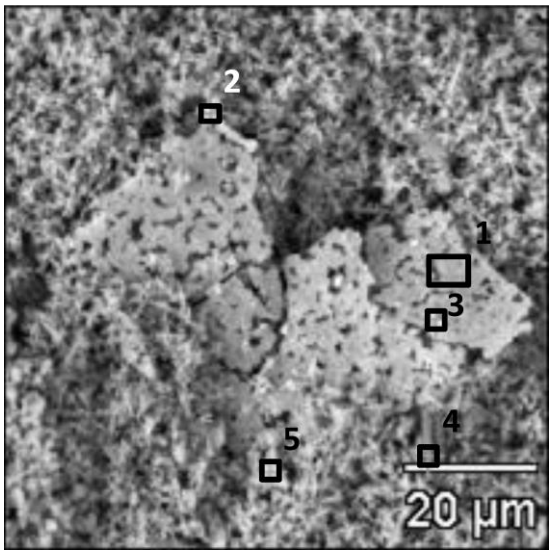
Table 7.36 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.37 determined using EDS

Sample_Area	Atomic %[1]											
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Fe**	**Ni**	**Cu**	**Zn**
UF-GU-filter(1)_ar1	-	-	-	0.9	-	99.1	-	-	-	-	-	-
UF-GU-filter(1)_ar2	-	-	0.7	4.2	1.3	84.8	-	3.8	1.4	0.8	-	3.0
UF-GU-filter(1)_ar3	0.9	-		2.6	-	91.7	-	2.4	-	-	-	2.4
UF-GU-filter(1)_ar4	-	0.1	1.0	20.5	1.6	65.1	0.4	2.9	1.2	0.8	1.1	5.4
UF-GU-filter(2)_ar1	1.4	-	6.3	34.7	-	52.0	4.7	-	0.9	-	-	-
UF-GU-filter(2)_ar2	1.5	6.0	8.1	19.4	-	50.5	-	12.1	1.8	-	-	0.6
UF-GU-filter(2)_ar3	-	-	-	-	-	100.0	-	-	-	-	-	-
UF-GU-filter(2)_ar4	-	-	-	10.7	-	88.8	-	0.5	-	-	-	-

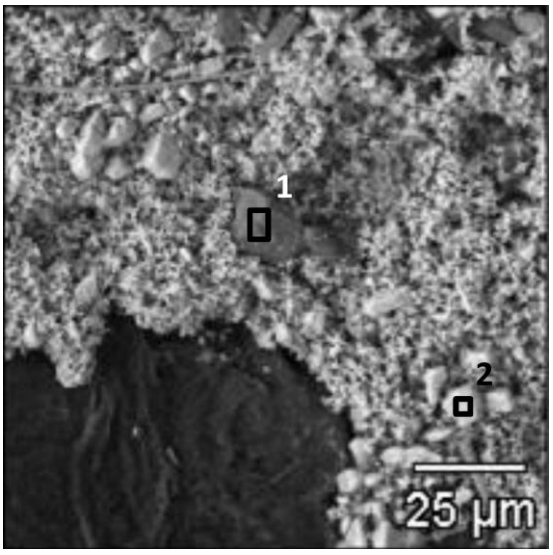
[1]Compositions normalized to 100%

It can be seen that all the analysed areas contained high amounts of S. It was mostly in amorphous form but could have also been present as in the crystalline form (area 1 of sample UF-GU-filter(2)). Also the metals of concern including Ni, Cu and Zn were present on the filter as species that are difficult to determine, however, the presence of sulphides cannot be excluded.

Another two areas on the filter were analysed to provide more information on the elements and species that were retained. The SEM-BSE images of the filter through which the wastewater form the UF-GU column was filtered are presented in Fig. 7.38 and the elemental composition of the selected areas are listed in Tab. 7.37.



(a) Sample UF-GU-filter(3)



(b) Sample UF-GU-filter(4)

Figure 7.38 SEM-BSE images of the filter surface through which the effluent from the UF-GU column was passed (system B)

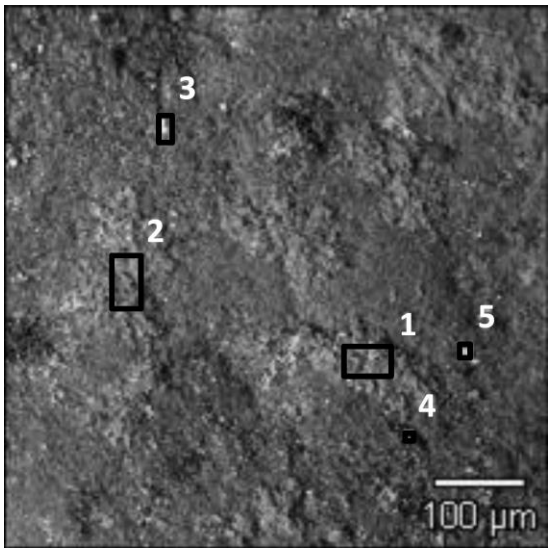
Table 7.37 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.38 determined using EDS

Sample_Area	Atomic %[1]											
	Na	**Mg**	**Al**	**Si**	**S**	**K**	**Ca**	**Fe**	**Ti**	**Ni**	**Cu**	**Zn**
UF-GU-filter(3)_pt1	-	-	-	1.3	85.8	-	-	-	-	0.8	5.4	6.7
UF-GU-filter(3)_pt2	-	-	-	1.9	83.3	-	1.5	-	-	1.1	5.9	6.4
UF-GU-filter(3)_pt3	-	-	-	1.3	88.0	-	0.4	-	-	-	4.2	6.2
UF-GU-filter(3)_pt4	4.1	0.3	3.0	24.9	62.0	1.0	1.0	-	0.7	-	-	3.1
UF-GU-filter(3)_pt5	-	-	-	0.5	99.5	-	-	-	-	-	-	-
UF-GU-filter(4)_pt1	0.3	-	2.3	39.9	53.7	0.4	0.4	1.2	-	-	-	1.7
UF-GU-filter(4)_pt2	-	-	-	1.0	99.0	-	-	-	-	-	-	-

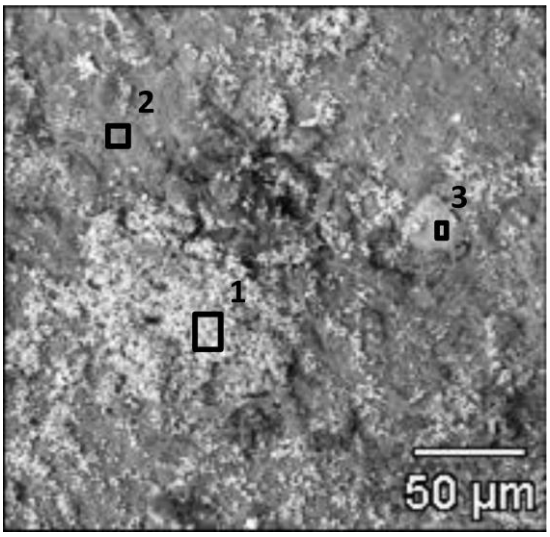
[1]Compositions normalized to 100%

In general the findings for the areas presented in Fig. 7.38 are similar to those presented above for the same filter. In the UF-GU-filter(4) sample more crystals of sulphur were observed. The metals of concern, especially Cu and Zn were present in higher concentration that in the previous two samples.

The white tarnish was also observed on the topmost grains of gravel in the UF-GU column. This layer of gravel was collected and analysed to confirm if the white precipitates found on the filter were also present on the gravel. The SEM-BSE images of topmost gravel from the UF-GU column are presented in Fig. 7.39 and the elemental composition of the selected areas is listed in Tab. 7.38.



(b) Sample UF-GU-top(1)



(b) Sample UF-GU-top(2)

Figure 7.39 SEM-BSE images of wetland substrates sampled from the top of the UF-GU column (system B)

Table 7.38 Elemental (atomic %) composition of the areas (ar#) indicated in the images in Fig. 7.39 determined using EDS

Sample_Area	Atomic %[1]										
	Na	**Mg**	**Al**	**Si**	**P**	**S**	**K**	**Ca**	**Ti**	**Fe**	**Ni**
UF-GU-top(1)_ar1	2.3	0.1	4.6	25.3	-	40.8	1.1	24.6	-	0.7	0.5
UF-GU-top(1)_ar2	2.6	-	5.0	25.8	-	36.5	0.9	28.4	-	0.9	-
UF-GU-top(1)_ar3	7.1	-	13.5	62.6	-	8.8	3.5	2.2	1.3	1.1	-
UF-GU-top(1)_ar4	5.1	0.5	12.1	59.9	0.8	12.1	3.9	3.5	-	1.3	0.7
UF-GU-top(1)_ar5	4.3	0.5	9.5	61.3	-	10.6	2.6	2.8	-	8.4	-
UF-GU-top(2)_ar1	1.2	-	2.5	15.4	-	80.0	0.5	0.4	-	-	-
UF-GU-top(2)_ar2	4.0	0.6	12.4	56.6	-	19.5	4.1	0.7	-	2.2	-
UF-GU-top(2)_ar3	2.5	3.0	12.6	44.3	-	21.3	5.0	-	1.2	10.0	-

[1]Compositions normalized to 100%

The EDS analysis of the gravel confirmed the presence of S, however, in quantities smaller than on the filter. This could have been be caused, amongst others, by the composition of the gravel itself and the drying of gravel, which could have promoted precipitation of sulphates of, for example, Ca and Na. However, most of the forms observed on the gravel are not crystalline, whereas sulphates of Ca and Na form crystalline forms. It is noticeable that the amount of the S precipitates is smaller than on the filter and that it forms clusters of different sizes. The largest cluster of S (area 1 of sample UF-GU-top(2)) contained 80% of this element. The crystalline forms in sample UF-GU-top(1) that had the appearance of a rosette consisted mainly of S and Ca and were probably gypsum.

It should also be mentioned that the white precipitates observed in the UF-GU column cannot be construed as S with absolute certainty. This is because some of the heavy metals have X-ray spectra that overlap the light element X-ray spectra and create difficulties with analysis. In this

study it is of particular importance that the M series of Pb at 2.346 and 2.502 overlaps the Kα line of S (Echlin, 2009). Similar appearance as is assumed to be caused by elemental S can be also caused by the precipitates of Pb sulphate. Hence, the hypothesized presence of sulphur should be confirmed using other analytical method.

The unsightly appearance of the dilute skim milk phenomena, whether it is elemental S or not, should be prevented in the full-scale systems.

7.8.9 Discussion of the sequential chemical extraction and SEM-EDS analyses of the substrates

It was observed that the intended removal of metals in the form of sulphide precipitates was not a major removal for most of the column configurations and elements. It was also found that the observed clusters of sulphides were containing Fe more frequently than the other metals of concern. Lead was not detected by the SEM-EDS analysis because of peak overlapping with S (details in Section 7.8.8). The observations made for the reported experiments were previously discussed in the literature. Two the most representative studies are discussed below. The findings of Neculita et al. (2008b) based on the experiment in a microcosm DF columns indicated that only 15% of total metals were removed as sulphides. In that experiment 3.5 L columns were fed with simulated acid mine drainage with Ni and Zn concentration in the range of 13-15 mg/L, 4 g/L of sulphate and acidic pH. Their findings suggested that the major removal of metals was attributed to adsorption or complexation with organic matter and co-precipitation as (oxy)hydroxide minerals. The duration of their experiment (44 weeks) (Neculita et al., 2008a) was shorter than the experiments described in this thesis. Similarly to this study Neculita et al. (2008b) used SEP and the SEM-EDS analysis to analyse the substrates sampled from the columns. The additional method that was applied by these authors was determination of acid volatile sulphides-extractable metals, which yields the ratio between the metals bound with sulphides and the volatile sulphides. The ratio between extractable metals and acid-volatile sulphides determined by these authors was much greater than 1 indicating high mobility of metals in acidic conditions. This analysis was not applied in this study but the similarity of the other results allows for hypothesis that the mobility of metals in the investigated systems should be a matter of concern. Song (2003) found that Zn and Pb sulphides were sparse and randomly scattered in the substrate of microcosm CW fed with simulated mine drainage water containing 50 µg/L Pb, 0.5 mg/L Zn, 0.23 mg/L Fe, 34.2 mg/L SO_4^{2-} and with pH ca. 8. This system was operated for 4 years. Moreover, Song (2003) stated that SEM-EDS analysis might not be sufficient to observe metal sulphides at low concentrations present in the investigated CW. The

increased affinity of Fe for sulphides was also reported by Song (2003) who elucidated that binding of Fe with sulphides is more thermodynamically favoured as compared to Pb or Zn, for example.

Interestingly, it was reported by Obarska-Pempkowiak and Klimkowska (1999) that the dominant metal removal mechanism in a hybrid CW treating domestic wastewater was adsorption to particulate suspended matter. The concentration of metals in the domestic wastewater was, however, much lower than in the mine drainage or industrial wastewater, and the amount, of organic matter, on the other hand, was higher (Obarska and Klimkowska, 1999; Obarska-Pempkowiak, 2001).

7.9 Contaminants in plants in system A

The concentration of metals in above- and belowground biomass of *Phragmites australis* are shown in Table 7.39. The sampled biomass was taken from the FaD columns fed with both types of wastewater in month 12 of the experiment (May, 2012) and the concentration of metals and B in the above- and belowground biomass of *Phragmites australis* was analysed. The ratio between the element concentration in the above- and belowground biomass of the plants is translocation factor (TF), which indicates translocation of element into the aboveground parts of the plants (Deng et al., 2004; Lesage 2006). Similarly, the bioconcentration factors (BCF) is a ratio between metal (or B) concentration in the belowground part of plants and metal concentration in the substrate (here exchangeable fraction in the top part of the substrate as determined by the SEP, not included in Tab. 7.39) (Lesage, 2006; Yadav et al., 2012). The TF and BCF values are also given in Tab. 7.39.

Table 7.39 Concentration of metals and boron (mg/kg DW) in aboveground (Above) and belowground (Below) parts of the plants and the translocation factors (TF) and bioconcentration factor (BCF) for the FaD columns

Column	Part/Factor	Al	B	Cu	Fe	Mn	Ni	Zn
		Cyanide wastewater						
FaD-CP	**Above**	40.402	40.06	11.415	123.285	62.441	5.957	35.064
	Below	71.039	21.50	14.230	241.611	65.186	4.651	28.658
	TF	0.57	1.86	0.80	0.51	0.96	1.28	1.22
	BCF	2.02	1.67	1.76	6.71	3.61	0.25	4.15
FaD-SP	**Above**	38.432	44.41	8.470	87.216	30.274	5.944	19.042
	Below	918.043	83.77	27.362	2 399.564	46.454	17.691	31.574
	TF	0.04	0.53	0.31	0.04	0.65	0.34	0.60
	BCF	48.39	17.40	1.22	38.93	16.24	0.97	10.60
		Feed without cyanides						
FaD-CP	**Above**	33.129	41.48	7.497	115.049	41.257	4.230	40.574
	Below	41.708	22.35	5.874	176.220	36.004	3.281	36.374
	TF	0.79	1.86	1.28	0.65	1.15	1.29	1.12
	BCF	1.13	0.79	10.22	8.91	1.80	1.84	5.06
FaD-SP	**Above**	29.275	33.23	5.792	100.345	30.993	3.948	19.862
	Below	137.498	22.20	9.200	328.620	33.296	3.982	23.108
	TF	0.21	1.50	0.63	0.31	0.93	0.99	0.86
	BCF	4.87	3.40	10.87	20.91	6.80	2.67	2.88

The order of concentration of metals in the aboveground biomass of *Phragmites australis* was Fe > Mn > Al > Zn > Cu > Ni, and was similar to the order in belowground parts, in which Al concentration was higher than that of Mn. Iron concentration in either parts of plants was at least 2-fold higher than concentration of Mn or Al. It can be seen that the TFs for Al and Fe were lower than 1 for all the FaD columns. It was also observed that the TF is lower than 1 for all the metals in the FaD-SP columns. For comparison, the TF was higher than 1 for Ni, Cu, Zn and Mn in one or two FaD-CP columns (depending on the feed). This suggests that the substrate could have played a role in the transport of metals to the aboveground parts of the plants. Differently than for metals the TF for B was higher than 1 (with the exception of the FaD columns fed with cyanide wastewater), which probably stems from the fact that B is an essential micronutrient for plants and plays important role in the cell walls in the plants and in the metabolism of carbohydrates. Usually, the concentration of B is higher in the aboveground parts of the plants, especially in older leaves (Kabata-Pendias and Pendias, 1999). Zhu et al. (1999) stated that a good accumulator must have the capability of accumulating more than 5000 mg/kg DW (0.5%) and bioconcentrating metals with the BCF value higher than 1000. Neither of these conditions were met and in general were much lower than the threshold values. This implies that *Phragmites australis* did not play a major role as an accumulator of metals.

The concentrations of metals and B determined in the plants samples were comparable with the values reported in the literature mainly for various natural and constructed wetlands (Tab. 7.40).

Table 7.40 Metal and boron concentration (mg/kg DW) in the above- and belowground biomass of *Phragmites australis* reported in the literature

Element	Plant part	
	Aboveground	**Belowground**
Al	31[1]-185[2]	102[1]-4228[2]
B	2.96[2]	3.20[2]
Cu	6.98[2]-59[9]	9.2[7]-32.8[2]
Fe	76[2]-1053[3]	2084[2]-9280[4]
Mn	28[5]-265[4]	62[5]-567[4]
Ni	1.33[2]-8.4[6]	4.59[2]-9.4[8]
Zn	23.6[2]-372[7]	31.6[2]-372[7]

[1]Peverly et al. (1995), CWs; [2]Vymazal et al. (2009), CWs; [3]Samecka-Cymerman and Kampers (2001), natural wetlands; [4]Eckhardt et al. (1999), CWs; [5]Behrends et al. (1996), CWs; [6]Vymazal and Krasa, (2005); CWs; [7]Lesage (2006), CWs. [8]Lesage et al. (2007), CWs; [9]Ye et al. (2003), plants collected at mine sites.

The analysed concentration of metals in the common reed is in the range or even below the concentrations reported in the literature (Tab. 7.40). The exception is the Ni concentration in the belowground parts of the FaD-SP column fed with the cyanide wastewater. The concentration of B was found to be markedly higher, by an order of magnitude, in both below- and aboveground parts of the common reed.

As it was pointed out by Lesage (2006) the metal concentrations in the belowground biomass of the common reed presumably include metals that were adsorbed or co-precipitated (or both) on the Fe-(oxy)hydroxide plaques covering the root surface. This could result from the methodology of preparation of the plants for the analysis, which were rinsed only with deionised water. Major part of metals can be bound to the below-ground parts of plants as co-precipitates in the root plague (Gries and Garbe, 1989; Mays and Edwards, 2001; Lesage, 2006).

Pearson correlation test was used to determine correlation between metal concentration in substrate (exchangeable fraction) and above- and belowground parts of *Phragmites australis* of the FaD columns of system A (Table 7.41). The exchangeable fraction of metals and B as determined by the use of the SEP (Rauret et al., 1999) and is believed to be plant-available (Bacon and Davidson, 2008).

Table 7.41 Pearson correlation coefficients (r) between metal concentrations in the top layer of substrate (TopS), belowground biomass (BLG), and aboveground biomass (ABG) of *Phragmites australis,*

Sampling point	Al	B	Cu	Fe	Mn	Ni	Zn
	r[1]						
TopS *and* ABG	-0.10	0.20	0.37	-0.51	**0.76**	**1.00**	0.43
TopS *and* BLG	-0.93	-0.51	**0.99**	**0.90**	0.31	**0.61**	-0.33
BG *and* ABG	0.36	**0.65**	0.32	-0.83	**0.82**	**0.62**	**0.62**

[1]at $p < 0.05$; strong positive correlations (above 0.5) are shown in bold

A strong positive correlation between metal concentration in top substrate layer and aboveground biomass of plants was observed for Mn (r = 0.76) and Ni (r = 1.00), and moderate positive correlation for Cu (r = 0.37) and Zn (r = 0.43). The correlation between concentration of metals in the top layer of substrate and belowground biomass of plants was strong for Ni (r = 0.66), and Fe (r = 0.90) and Cu (n = 0.99) and moderate for Mn (r = 0.31). Metal concentration in the above- and belowground biomass of plants was strongly positively correlated for Ni (r = 0.62), Zn (r = 0.62), B (r = 0.65), Mn (r = 0.82), and moderately for Cu (r = 0.32) and Al (r = 0.36). The interpretation of the results may be difficult as the analysis of the elemental concentration of plants was determined only once after one year after the planting. The strong negative correlation (r = -0.93) between the Al concentration in the substrate and the belowground parts of the plants may suggest that the roots of common reed subjected to elevated concentrations of Al exuded phosphates that complexed Al^{3+} limiting its uptake (Pendias-Kabata and Pendias, 1999). The correlation data for B imply that this element was transported from the roots and rhizomes of the plants to the aboveground parts. It can probably also be observed that B was already stored in the seedling of the plants before they were planted in system A or that majority of B was transported from the belowground parts to the aboveground tissues for their growth. The data in Tab. 7.41 may suggest that Cu was taken by plants and then was transported to the aboveground parts. This is in agreement with the literature data that Cu is taken by roots and translocated to the offshoots (Obarska-Pempkowiak et al., 2010). The strong correlation between the Fe concentration in the substrate and the belowground parts of the plants indicate that the uptake of this metal was dependent on the concentration in the substrate. The negative correlation between the Fe concentration in the analysed parts of the plants and the substrate and the plants' aboveground concentration may suggest that the Fe translocation the aboveground biomass of the common reed was independent or even partly inhibited by increased concentration of Fe or other metals. The correlation results in Tab. 7.41 imply that Mn was translocated from the below- to the aboveground parts of plants and that it was probably stored in the plants before the planting or at the initial stage of the experiment. It can be observed that

Ni was taken up by the plants and evenly distributed between the analysed parts of the common reed. The fate of Zn in *Phragmites australis* was similar to the fate of B, which is indicated by the correlation data (Tab. 7.41).

7.10 Oxidation-reduction potential in the constructed wetlands

The ORP was measured several times in the end of the experiment in system A. The mean values are given for the water layer and the 10-cm-thick top and bottom parts of the substrate in the FaD columns (Fig. 7.40).

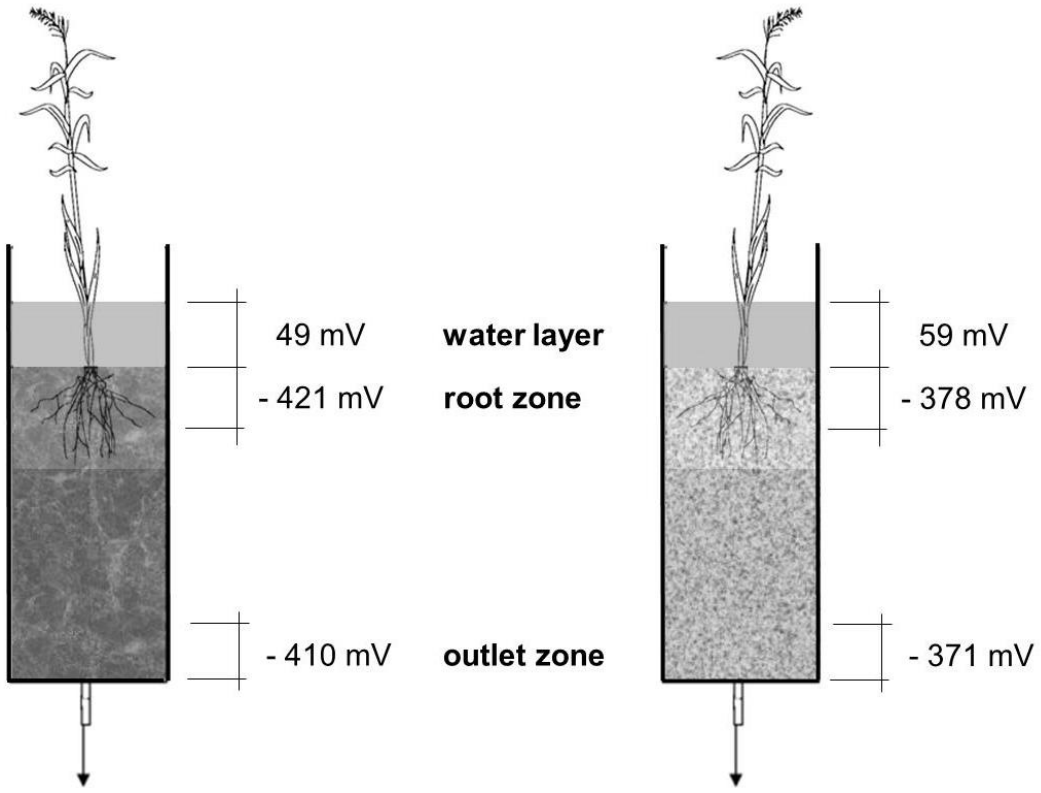


Figure 7.40 Oxidation-reduction potential in the substrate and water layer of the FaD-CP (left), FaD-SP (right), system A

The conditions in the substrate of the FaD columns were strongly reducing and the ORP was below the threshold allowing for methanogensis (-200 mV). The columns with organic bed media had slightly lower ORP of the substrate and water layer. It was also observed that the ORP in the root zone was slightly lower that the effect in transporting oxygen from the above ground parts was negligible, however, the measurement were not made in the close proximity to the roots. In these redox conditions SRB and methanogens may compete for electron donors. Interestingly, approximately 14% of methanogens may use acetic acid, which is used by SRB but

also produced by the lactate-oxidizing SRB. Both groups of bacteria may also compete for dimethyl sulphide, which is abundant in anoxic wetlands (Paul and Clark, 1996; Vymazal and Kröpfelová, 2008). The emission of methane and other gases from the system were not quantified, however, this negative aspect should be considered when planning full-scale implementation. It should also be mentioned that the measurements were made in the end of a two-week batch suggesting that this duration is suitable to provide reducing conditions in the bed and thus limit oxidation and release of the reduced forms of metals, particularly sulphides.

7.11 Additional aspects of system operation

7.11.1 Disposal of the spent substrate

Little information is available on the possible fate of spent reactive mixtures, except for the fact they are sometimes replaced/recharged because they become inefficient (Doshi, 2006). Incineration of the spent substrate was considered but its properties disallow this treatment, especially because of the necessity for separation of the mineral fraction from the organic phase. It appears that gasification of the mixed organic-mineral substrate may be a viable solution, which will be studied using the substrate from the described system B. There also physical-chemical methods that could be used for treating the spent substrates, e.g. using hydrogen peroxide, but their application was not well studied (Lesage, 2006). It is of immense importance to take appropriate action to prevent mobilisation of metals after the closure of a system. This could be done by simply maintaining waterlogged conditions in the system before any further treatment or excavation (Lesage, 2006).

7.11.2 Pest threat

During the summer months in the end of the experiment aphids were observed on the green leaves of *P. australis* in system A (Fig. 7.41). Upon closer inspection and literature study this genus of aphids was identified as the mealy plum aphid (*Hyalopterus pruni* (Geoffroy) (Horn.: Aphididae)). The mealy plum aphid is a pest of plum and apricot causing serious damage to orchards (Özgökçe and Atlıhan, 2005). The winged adults migrate in summer to common reed and other grasses. The mealy plum aphid produces honey dew (white droplets visible in the photo – Fig. 7.41), which may attract other insects (www.1). Noteworthy, no serious damage caused by the aphid was observed, however, this threat should not be neglected (www.1).

Figure 7.41 Aptera and nymphs of the mealy plum aphid (*Hyalopterus pruni*) on the leaves of *Phragmites australis* in system A (September, 2012)

7.11.3 Organisms observed in the experimental system

The identification of the organisms occurring in the experimental systems was not a goal of this study but the SEM analysis of the substrate from system B allowed observation of diatoms and *Daphnia*. Diatoms were observed on the filter through which the effluent from the UF-GU column was passed (Fig. 7.42 ab) and attached to the surface of gravel from the same column (Fig. 7.42 cd).

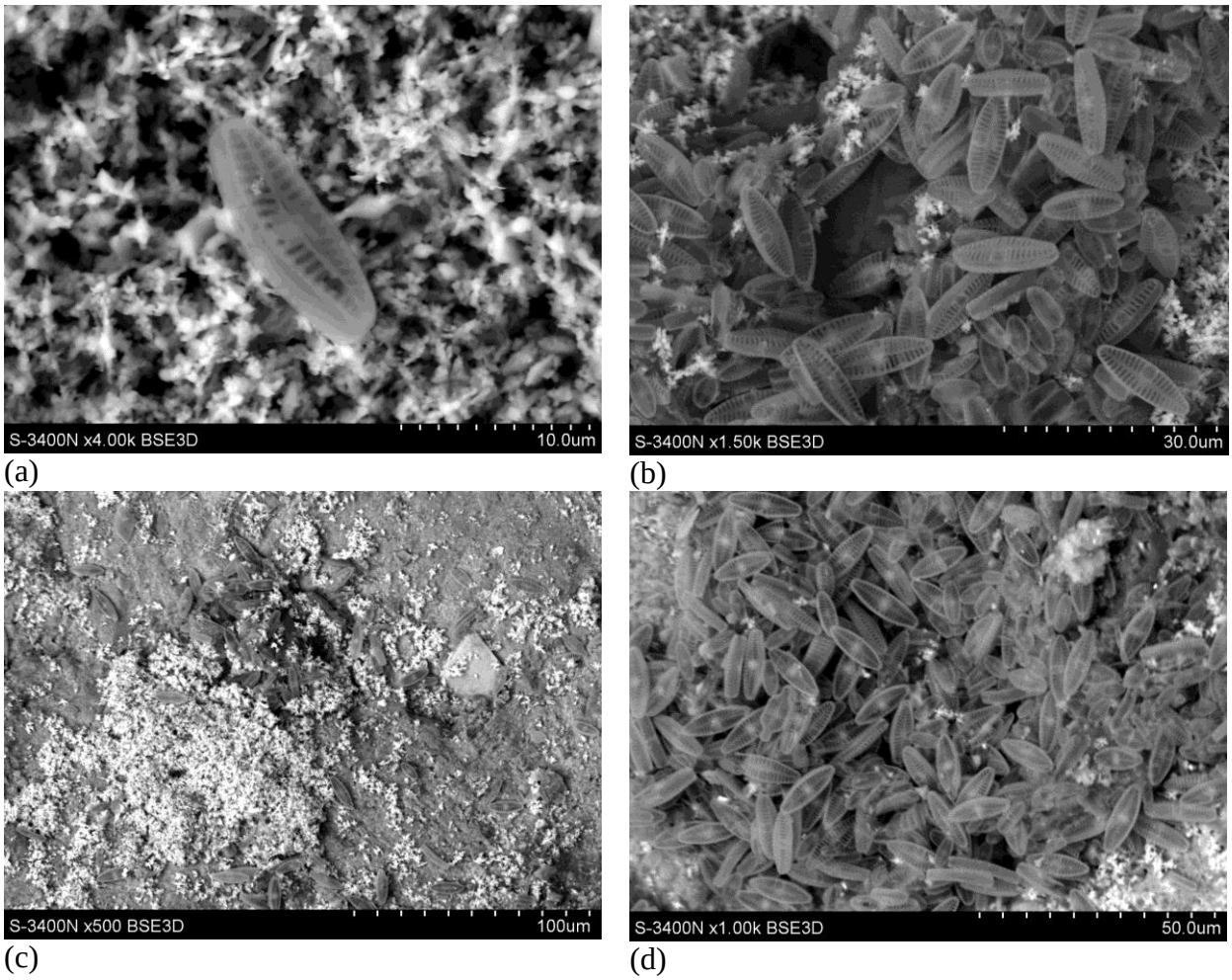
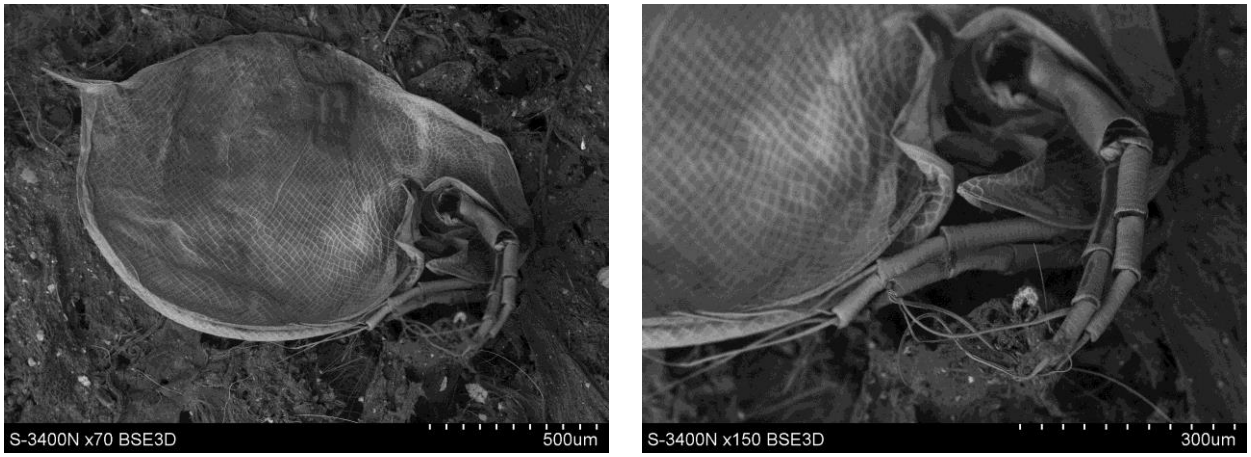


Figure 7.42 Diatoms observed (using SEM-BSE) on the filter (a, b) and attached to the surface of gravel (c, d) in the UF-GU column of system B

Daphnia was observed with the unaided eye in the columns with peat (UF-PP and UF-PU). Several *Daphnia* were observed on the filter used to filter the effluent from the UF-PP columns. An image of a single organism is shown in Fig. 7.43 ab.



(a) (b)

Figure 7.43 *Daphnia* spp. observed (using SEM-BSE) on the filter through which the water layer on top of the UF-PP columns was filtered (system B): a) *Daphnia* organism; b) *Daphnia* antenna

8 Design remarks and tentative calculations of a full-scale system

Out of several configurations that were tested during the experiments it appears that the UF-GP columns amended with external carbon source were the best solution for polishing of electroplating wastewater. The application of solely mineral substrate and continuous upward flow of wastewater provided high removal efficiency and the process reliability, however, not tested in long-term experiment. The risk of using organic media (e.g. compost or peat) is a release of organic matter, clogging, short-time expectancy of being a source of organic carbon for bacteria, and loss of adsorption properties. The addition of external carbon source is less economical but it provides more flexibility to the system by giving the possibility of controlling the amount of available carbon source and the resulting amount of produced sulphides. This is an important issue as an elevated concentration of sulphide may enhance metal solubility and compromise the performance of a treatment system (Gammons and Frandsen, 2001).

The results of a microcosm study cannot be directly extrapolated to a full-scale system. This was not attempted in this study. The obtained results for the UF-GP columns were only used to present how the microcosm data can be used for tentative calculations of a full-scale system to be implemented at the premises of the PEM company to treat the effluent of its electroplating plant. According to Kadlec and Wallace (2009) two the most common approaches to design of the CWs for the treatment of wastewater containing metals are based on the following equations:

- the first approach:

$$R_A = \frac{Q}{A}(C_i - C_o) \tag{8.1}$$

- and the second approach:

$$\frac{C_o}{C_i} = \exp\left(\frac{kA}{Q}\right) \tag{8.2}$$

Where : A = wetland area, m^2; C_i = inlet concentration, mg/L; C_o = outlet concentration, mg/L; Q = flow rate, m^3/d; R_A = area-adjusted contaminant removal rate, g/m^2d; k = areal rate constant, m/d.

The first approach was also recommended in the PIRAMID Guidelines (2003) as a sizing formula for compost CWs.

One of the goals of the presented microcosm study was to test the feasibility of using CWs for polishing of the wastewater byproduced at the PEM. The full-scale system could be

implemented, after pilot-scale studies, at the premises of the PEM company. Figure 8.1 presents a picture of one of two ditches, situated at the premises, which could be used for construction of a CW system treating the effluent from the company. The total area of these two ditches is about 1450 m^3.

Figure 8.1 Potential treatment site at the premises of the PEM in Siaugues-Sainte-Marie, France

The R_A was calculated (using eq. 8.1) based on the data from weeks 44−53 for the UF-GP columns fed with the high-strength influent amended with lactate (Tab. 8.1).

Table 8.1 Input data for the calculation of required area for the full-scale CW

	C_{in} **g/m^3**	C_{out} **g/m^3**	**Q m^3/d**	**A m^2**	**R g/m^2 d**
Pb	0.78	0.25	$2.88 \cdot 10^{-3}$	0.0314	0.05
Cu	2.65	0.04			0.24
Ni	5.83	0.12			0.52
Zn	6.32	0.48			0.54

The results of the calculations suggest that the lowest value of the R_A was for Pb and this value should be considered in the design of a full-scale system. Knowing the R_A values it is possible to calculate the required area of a full-scale system that would have the same configuration properties as the studied UF-GP columns. The data in Tab. 8.2 present the quality of the real

electroplating effluent from the PEM company (of April 2012; June 2008 for Pb) and the target effluent concentrations, which are the detection limits for these elements of the ICP-AES apparatus used in this study. Based on the R_A value (Tab. 8.1) and the desired treatment effect the required area of a CW was calculated along with corresponding HRT (d) (Tab. 8.2). The HRT value was calculated based on the assumptions that the full-scale system will have the same water depth and porosity as the experimental UF-GP (h = 0.7 m, ε = 0.4, respectively)

Table 8.2 Area requirement based on the calculated area-adjusted contaminant removal rate (R_A)

	C_{in}	C_{out}	Q^1	R_A	A	HRT
	g/m^3	g/m^3	m^3/d	$g/m^2 \cdot d$	m^2	d
Pb	0.05	0.015	380	0.05	273.6	0.20
Cu	0.095	0.001	380	0.24	149.2	0.11
Ni	0.069	0.002	380	0.52	48.6	0.04
Zn	0.109	0.001	380	0.54	76.6	0.06

[1]typical flow rate of the wastewater treated at the PEM

It can be seen that the required area for the UF-CW is much smaller than the available one (273.6 and 1450 m^2, respectively). This is however doubtful that the desired treatment effect can be achieved as the HRT values are much lower than in the experimental UF-GP. If the whole available area would be used for constructing the CW the corresponding HRT value would be about 4 d.

9 General discussion and conclusions

This study furnished new information and expanded the existing knowledge on industrial wastewater treatment in CWs. In fact, this is, to the best knowledge, the first publication studying the removal of metals and cyanide from electroplating wastewater in subsurface flow constructed wetlands. The aim of this thesis was to study the polishing of the electroplating wastewater pretreated using physical-chemical methods.

The experiment was performed in two experimental systems that differed in many aspects, therefore meaningful inter-experimental comparison cannot be made. Thus, it is critical to study the performance of the future full-scale system in a micro- or mesocosm model onsite using the real wastewater. One of the challenges of polishing electroplating wastewater is that it contains low concentrations of metals and is deficient in organic matter. This relationship was observed in both systems and also when the two systems were compared with each other.

High removal efficiency can be achieved for all the metals of concern: Al, Cu, Ni, Zn, save Pb, depending on the influent quality and the configuration of the CW. From the operational perspective only the UF columns are worth considering for application on full-scale. This is because the FaD-CWs are more erratic in operation and have the propensity for clogging when the organic bed media (peat or compost) are used.

Cyanide removal is more efficient in the CWs with constantly anoxic conditions (UF columns) than in the CWs with cycling oxidizing and reducing phases (FaD columns).

Boron can be removed only in the CWs with internal carbon source such as compost. It is a conservative element when the wastewater is treated in the CWs with mineral bed media.

Higher hydraulic loading rate allows for higher removal efficiency of metals in the UF columns. In contrast to the UF columns, longer holding phase in the FaD columns corresponds to higher removal efficiency of metals.

The removal of metals and sulphate can be increased when lactate as an external carbon source is added to the influent to stimulate the activity of SRB. The use of lactate allows achieving higher treatment efficiency than in the counterpart CWs with the internal carbon source and comparable effluent concentration of organic matter.

The FaD CWs may be used as a buffer in emergency events to prevent pollution of the environment from the raw electroplating wastewater. In this situation the CWs may remove

some of the metallic contaminants, decrease concentration of cyanide and markedly attenuate extreme alkaline pH of the electroplating wastewater. The discharge of aggressive electroplating wastewater may exert and adverse effect on the subsequent performance of the CW relative to certain contaminants.

The role of plants in the removal of metals, B and cyanide was minor. Moreover, the observed effect of the presence of vegetation on the treatment efficiency was different for the metallic contaminants, thus it is difficult to draw definite conclusion. It should be emphasized that plants played a role preventing algal bloom and offering aesthetical benefits to the CW.

The intended process for removal of metals was precipitation of metals with biogenic sulphide being the product of the activity of SRB. This process was found not to play a major role in removal of metals. The major portion of metals was bound to exchangeable or reducible fractions. It allows conclusion that adsorption and oxic removal processes are responsible for the removal of metals in a short-time experiment. These processes are not recommended because metals can be easily leached from these phases upon change of process conditions. The commonly available tools as SEP and SEM-EDS analysis, however, tend to underestimate the contribution of the metal sulphide precipitation. The influent-effluent comparison of the S species suggested that the amount of produced sulphide was capable of scavenging the metals in the system. Further experiments and additional methods should be used to explain these phenomena.

10 Scope for further research

The experiment reported in this did not address all the interesting issues of polishing electroplating wastewater in CWs. Some of them were not deliberately targeted in this research and some of them appeared during the experiment. There are in fact many aspects of this research that could be debated in terms of improvement or scientific exploration. Only several of them are shortly discussed below.

Long-term and full-scale study of the electroplating wastewater polishing

Short-term studies and microcosm studies would not represent the treatment performance of a full-scale CW. Microcosm CWs can be subjected to severe edge effects and do not show the full suite of components of a fully developed CW (Kadlec and Wallace, 2009). Also this study did not assess the maturation of a CW polishing electroplating wastewater. It was, for example, observed that the metal sulphides precipitation was not the dominant removal processes as the other processes played a major role. It is however expected that the precipitation of sulphides increases its contribution with time when the adsorption sites on the substrate become depleted (Wallace and Knight, 2006). This hypothesis should be studied for the electroplating wastewater. Further study and pilot-scale implementation of a CW in co-operation with the PEM company will be considered.

Microbiological investigation of the bacterial community in the CWs

The presence of SRB in the investigated CWs was only based on indirect evidence as the reduction of sulphate concentration and the fact that the inoculum was used. Direct methods should be used to assess the density, diversity and activity of the bacteria present in the CWs. Knowing how the controllable factors affect the bacterial community would allow for optimization and better design of CWs tailored to removal of particular group of contaminants (Faulwetter et al., 2009). Substrate samples from system A and B will be subjected to microbiological analysis and the results will be discussed in further publications.

Using external carbon source alternative to lactic acid

It was found in this thesis that lactate as the external carbon source may improve markedly the removal of metals and sulphate in the CW. Lactic acid is, however, an expensive material. It was calculated based on the prices of lactic acid given in Wee et al. (2006) that a unit price of treating electroplating effluent with the ratio COD/SO_4^{2-} and the concentration of sulphate about 800 mg/L would be 0.35 Euro/m^3. The alternative source of carbon for the treatment of electroplating

wastewater can be waste material as dairy wastewater. The feasibility of using dairy wastewater as a source of carbon should be studied. Also the optimum ratio between electroplating and dairy wastewater should be determined to avoid the presence excessive organic substance in the effluent. The dairy wastewater apart from organic matter contains phosphorus and ammonia nitrogen that could stimulate the growth of plants. Wallace and Knight (2006) reported the BOD_5 value of dairy wastewater to be about 2700 mg O_2/L. Assuming that all the BOD_5 is available for SRB it was calculated that the ratio between the dairy wastewater and the electroplating wastewater (as studied in the experiment) should be 0.1−0.17. Thus, for example to treat 380 m^3 of the electroplating wastewater at the PEM company about 65 m^3 of the dairy wastewater would be required a day.

Using hybrid system with aerobic CW

In an effectively operating compost CW the effluent often has a low dissolved oxygen concentration, and therefore it is normal practice to follow a compost CW with an aerobic system. This system oxygenates the wastewater, removes residual iron and aluminium as hydroxides and co-precipitates other metals (PIRAMID, 2003). This secondary step should be considered when designing the full-scale CW for the polishing of electroplating wastewater

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École Nationale Supérieure des Mines

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DOCZYSZCZANIE ŚCIEKÓW GALWANIZERSKICH W OCZYSZCZALNIACH HYDROFITOWYCH

Specjalność: Inżynieria środowiska

Słowa kluczowe: oczyszczalnie hydrofitowe; cyjanki; ścieki galwanizerskie; metale, doczyszczanie ścieków

Streszczenie:

Przemysł galwanizerski generuje dużą ilość ścieków, które zawierają znaczne ilości metali i cyjanków. Z tego powodu ścieki galwanizerskie mogą spowodować poważne zagrożenie dla środowiska, jeśli nie są prawidłowo oczyszczone. Oczyszczanie lub doczyszczanie ścieków galwanizerskich w oczyszczalniach hydrofitowych zostało opisane w literaturze w znikomym stopniu. To uniemożliwia sformułowanie jakichkolwiek wniosków dotyczących szerszego stosowania oczyszczalni hydrofitowych do doczyszczania ścieków podczyszczonych metodami fizyko-chemicznymi. Wysokie stężenia toksycznych zanieczyszczeń w surowych ściekach galwanizerskich uniemożliwiają stosowanie metod biologicznych do ich oczyszczania. Oczyszczalnie hydrofitowe nie stanowią więc alternatywy dla tradycyjnych fizyko-chemicznych metod oczyszczania ścieków galwanizerskich. Oczyszczalnie hydrofitowe mogą jednak służyć jako atrakcyjne uzupełnienie konwencjonalnych metod oczyszczania ścieków galwanizerskich przed ich odprowadzeniem do wód, ale także jako odbiornik awaryjny w przypadku usterki układu podczyszczającego ścieki.

Celem eksperymentu było: (i) zbadanie możliwości doczyszczania ścieków galwanizerskich, (ii) zbadanie wpływu różnych czynników na stopień usuwania zanieczyszczeń w układzie badawczym warunków eksploatacji układu, m.in. warunków eksploatacyjnych, obecności roślin, rodzaju złoża, stężenia zanieczyszczeń w ściekach (iii) określenie mechanizmu usuwania metali, w modelowej oczyszczalni hydrofitowej w skali laboratoryjnej.

Doświadczenie przedstawione w niniejszej pracy przeprowadzono w dwóch układach badawczych, z których każdy składał się z 12 kolumn. Głównym celem projektowania układu badawczego było wytworzenie w nim warunków sprzyjających usuwaniu metali (Cu, Ni, Pb, Zn) poprzez ich wytrącanie w postaci siarczków, w procesie w którym główną rolę odgrywają bakterie redukujące siarczany, które, na ogół, są na ogół obligatoryjnymi anaerobami. Z tego względu, większość z kolumn miało sprzyjać powstaniu i utrzymaniu warunków beztlenowych (8 z 12 kolumn w każdym systemie). Warunki beztlenowe w kolumnach były promowane poprzez ich budowę i sposób eksploatacji. Ścieki przepływały prze złoże tych kolumn w kierunku pionowym wstępującym. Ponad powierzchnią złoża znajdowała się 6,5-centymetrowa warstwa wody mająca zapobiegać przedostawaniu się powietrza do złoża. Ścieki do tych kolumn wprowadzono za pomocą pompy perystaltycznej przez króciec na dnie kolumny, a króciec odpływowy znajdował się 70 cm nad dnem. Dostępna literatura sugeruje, że metale i cyjanki mogą być również usuwane w warunkach tlenowych. W związku z tym, wybrane kolumny układu doświadczalnego były eksploatowane w sposób cyklicznie tlenowy i beztlenowy (4 z 12 kolumn w każdym systemie). Kolumny te był zasilane z okresowo w trybie “fill-and-drain”. Oba systemy doświadczalne zasilano rzeczywistymi lub syntetycznymi ścieków galwanizerskimi zawierających Al, B, Cu, Ni, Fe, Pb, Zn, cyjanki i siarczany jako zanieczyszczenia, których stopień usunięcia badano w trakcie eksperymentu.

Niniejsza rozpraw doktorska przedstawia różne aspekty doczyszczania ścieków galwanizerskich w oczyszczalniach hydrofitowych: analizę efektywności doczyszczania ścieków, określenie wpływu konfiguracji i sposobu eksploatacji układu na doczyszczanie ścieków, zbadanie wpływ różnych źrodeł węgla na stopień usunięcia zanieczyszczeń ze ścieków, analizę złoża, analizę roślin, sformułowanie wstępnych wytycznych projektowych.

Uzyskane wyniki doświadczeń dostarczyły nowe informacje i poszerzyły aktualną wiedzę na temat oczyszczania i doczyszczania ścieków galwanizerskich w oczyszczalniach hydrofitowych. Niniejsza praca jest prawdopodobnie pierwszą publikacją badającą usuwanie metali i cyjanków ze ścieków galwanizerskich w oczyszczalniach hydrofitowych o przepływie podpowierzchniowym.

Eksperyment przeprowadzono w dwóch układach doświadczalnych, które różniły się między sobą pod wieloma względami, dlatego miarodajne porównanie tych układów nie może być wykonane. Istotne jest, aby badać efektywność projektowanego układu w skali technicznej za pomocą modeli w skali ułamkowo technicznej w docelowej lokalizacji i przy użyciu rzeczywistych ścieków. Jednym z wyzwań doczyszczania ścieków galwanizerskich jest to, że

zawierają one niskie stężenie metali i są ubogie w materię organiczną. Zależność tę obserwowano w obu systemach, a także, gdy oba systemu były porównywane ze sobą.

Wysoką skuteczność usuwania można uzyskać dla wszystkich metali znajdujących się w badanych ściekach galwanizerskich tj.: Al, Cu, Ni, Zn, z wyjątkiem Pb, w zależności od właściwości nadawy i konfiguracji oczyszczalni hydrofitowej. Z eksploatacyjnego punktu widzenia jedynie kolumny o przepływie pionowym wstępującym kolumny są warte rozważenia pod względem zastosowania w skali technicznej. Kolumny typu "fill-and-drain" charakteryzują się znacznymi wahaniami skuteczności oczyszczania ścieków, poza tym ich złoże łatwiej ulega kolmatacji, w przypadku gdy jest częściowo wypełnione materiałem organicznym.

Stopień usunięcia cyjanków jest wyższy w oczyszczalniach hydrofitowych o niezmiennie beztlenowych warunkach niż w systemach o cyklicznie zmiennych warunkach tlenowo-beztlenowych.

Bor może być usunięty tylko w oczyszczalni hydrofitowej z wewnętrznym źródłem węgla, takim jak np. kompost. Pierwiastek ten nie jest zatrzymywany w złożu mineralnym.

Wyższa wartość obciążenia hydraulicznego pozwala na osiągnięcie wyższej skuteczności usuwania metali w kolumnach o przepływie pionowym wstępującym. Natomiast w kolumnach typu „fill-and-drain" dłuższa faza zatrzymania powoduje wyższy stopień usunięcia zanieczyszczeń.

Stopień usunięcia metali i siarczanów może zostać zwiększony przez dodanie mleczanów do nadawy, których obecność powoduje zwiększenie aktywności bakterii redukujących siarczany. Zastosowanie mleczanów umożliwia osiągnięcie większej skuteczności oczyszczania ścieków w kolumnach ze złożem mineralnym niż w kolumnach ze złożem organicznym, do których wprowadzano nadawę bez mleczanów.

Oczyszczalnie hydrofitowe typu „fill-and-drain" mogą być wykorzystane jako awaryjny odbiornik surowych ścieków galwanizerskich, w celu ograniczenia ich oddziaływania na środowisko. W tej sytuacji oczyszczalnie hydrofitowe mogą zmniejszyć stężenie niektórych metali w ściekach, zmniejszają stężenie cyjanków i znacznie obniżyć silnie zasadowe pH ścieków galwanizerskich. Wykorzystanie oczyszczalni hydrofitowych jako awaryjnego odbiornika surowych ścieków galwanizerskich może jednak wywrzeć niekorzystny wpływ na późniejszą efektywność doczyszczania podczyszczonych ścieków galwanizerskich w tych systemach.

Rola roślin w usuwaniu metali, boru i cyjanków była marginalna. Ponadto, zaobserwowano, iż wpływ roślin na usuwanie metali nie był jednoznaczny, dlatego trudno jest sformułować miarodajne wnioski. Należy podkreślić jednak, że rośliny odegrały posiłkową rolę w eksploatacji układu badawczego, ograniczając zakwit glonów i zwiększając estetykę modelu oczyszczalni hydrofitowej.

Pożądanym procesem usuwania metali, w opisanym eksperymencie, było ich strącanie w postaci siarczków, które są produktem aktywności bakterii redukujących siarczany. Stwierdzono jednak, że proces ten nie odegrał znaczącej roli w usuwaniu metali. Znaczna część metali była związana w złożu z frakcją wymienną lub redukowalną. To pozwala na sformułowanie wniosku, iż adsorpcja i tlenowe procesy usuwania metali są odpowiedzialne za ich usuwanie w przeprowadzonym, względnie krótkotrwałym, eksperymencie. Procesy te nie są pożądanym mechanizmem usuwania metali, ponieważ powstałe w ich wyniku fazy mogą w łatwy sposób ulec rozkładowi w przypadku zmiany warunków prowadzenia procesu, prowadząc do uwolnienia zatrzymanych metali. Powszechnie dostępne narzędzia lub procedury analityczne, jak sekwencyjna ekstrakcja chemiczna i SEM-EDS, mogą zaniżać rzeczywistą ilość siarczków w badanym złożu. Analiza form siarki w dopływie i odpływie z kolumn sugeruje, że ilość powstających siarczków byłaby odpowiednia do wytrącania zawartych w ściekach metali. Wykonanie dalszych eksperymentów i zastosowanie dodatkowych metod analitycznych mogłyby wyjaśnić to zjawisko.

École Nationale Supérieure des Mines

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ELECTROPLATING WASTEWATER POLISHING IN CONSTRUCTED WETLAND SYSTEMS

Speciality: Environmental Sciences and Engineering

Keywords: constructed wetlands; cyanide; electroplating wastewater; metals; wastewater polishing; wastewater treatment,

Abstract:

Electroplating industries generate a large volume of wastewater, which contains high amounts of metals and cyanides. Thus, electroplating wastewater may cause a serious threat to the environment if not treated properly. Electroplating wastewater treatment or polishing in constructed wetlands was studied to a very limited degree. This disallows any conclusions regarding broader application of constructed wetlands as a polishing step added to conventional treatment systems. High concentrations of toxic pollutants in the electroplating effluents prevent direct application of biological methods for their treatment. Thus, constructed wetlands are not an alternative to conventional physical-chemical methods of electroplating wastewater treatment. However, constructed wetlands may serve as a low cost add-on to conventional treatment of electroplating wastewater prior to its discharge into receiving waters but also as a buffer volume in case of failure of the upstream treatment facilities.

The goals of the experiment were to: (i) study the feasibility of polishing electroplating effluent, (ii) study the effect of operating schemes, plants, bed media and wastewater strength, (iii) to elucidate metal removal mechanisms in the constructed wetland microcosms.

The experiment was performed in two experimental systems consisting of 12 columns, each. The main objective of the system design was to promote metals (Cu, Ni, Pb, Zn) removal by precipitation as sulphides mediated by sulphate-reducing bacteria, which, in general, are obligate

anaerobes. Thus, most of the columns were designed to be strictly anaerobic (8 of 12 columns in each system). Anaerobic conditions in the columns were promoted by their construction and operation mode. These columns were operated in an upflow saturated mode with a 6.5-cm layer of water over the bed media preventing air from penetrating into the substrate. These columns were fed by a peristaltic pump through the bottom and the outlet was situated 70 cm above the bottom. However, also aerobic processes were reported in the literature to be efficient for removal of metals and cyanides. Therefore, selected columns of the experimental system were operated as intermittently anaerobic and aerobic (4 of 12 columns in the each system) which was enabled by the fill-and-drain mode of operation. The systems were fed with either real or synthetic electroplating wastewater containing Al, B, Cu, Ni, Fe, Pb, Zn, cyanides and sulphates as the contaminants of concern.

This thesis presents various aspects of polishing the electroplating wastewater in constructed wetlands: analysis of the performance, study on the operation and configuration effects, effects of various carbon sources, analysis of the substrate (bed media), analysis of plants, and design issues.

This study furnished new information and expanded the existing knowledge on industrial wastewater treatment in constructed wetlands. In fact, this is, to the best knowledge, the first publication studying the removal of metals and cyanide from electroplating wastewater in subsurface flow constructed wetlands. The aim of this study was to study the polishing of the electroplating wastewater pretreated using physical-chemical methods.

The experiment was performed in two experimental systems that differed in many aspects, therefore meaningful inter-experimental comparison cannot be made. Thus, it is critical to study the performance of the future full-scale system in a micro- or mesocosm model on the spot using the real wastewater. One of the challenges of polishing electroplating wastewater is that it contains low concentrations of metals and is deficient in organic matter. This relationship was observed in both systems and also when the two systems were compared with each other.

High removal efficiency can be achieved for all the metals of concern: Al, Cu, Ni, Zn, save Pb, depending on the influent quality and the configuration of the constructed wetland. From the operational perspective only the upflow columns are worth considering for application on full-scale. This is because the fill-and-drain columns are more erratic in operation and have the propensity for clogging when the organic bed media are used.

Cyanide removal is more efficient in the constructed wetlands with constantly anoxic conditions than in the constructed wetlands with cycling oxidizing and reducing phases.

Boron can be removed only in the constructed wetlands with internal carbon source such as compost. It is a conservative element when the wastewater is treated in the constructed wetlands with mineral bed media.

Higher hydraulic loading rate allows for higher removal efficiency of metals in the upflow columns. In contrast to the upflow columns, longer holding phase in the fill-and-drain columns corresponds to higher removal efficiency of metals.

The removal of metals and sulphate can be increased when lactate as an external carbon source is added to the influent to stimulate the activity of sulphate-reducing bacteria. The use of lactate allows achieving higher treatment efficiency than in the counterpart constructed wetlands with the internal carbon source and comparable effluent concentration of organic matter.

The fill-and-drain constructed wetlands may be used as a buffer in emergency events to prevent pollution of the environment from the raw electroplating wastewater. In this situation the constructed wetlands may remove some of the metallic contaminants, decrease concentration of cyanide and markedly attenuate extreme alkaline pH of the electroplating wastewater. The discharge of aggressive electroplating wastewater may exert an adverse effect on the subsequent performance of the constructed wetland relative to certain contaminants.

The role of plants in the removal of metals, boron and cyanide was minor. Moreover, the observed effect of the presence of vegetation on the treatment efficiency was different for the metallic contaminants, thus it is difficult to draw definite conclusion. It should be emphasized that plants played a role preventing algal bloom and offering aesthetical benefits to the constructed wetland.

The intended process for removal of metals was precipitation of metals with biogenic sulphide being the product of the activity of sulphate-reducing bacteria. This process was found not to play a major role in removal of metals. The major portion of metals was bound to exchangeable or reducible fractions. It allows conclusion that adsorption and oxic removal processes are responsible for the removal of metals in a short-time experiment. These processes are not recommended because metals can be easily leached from these phases upon change of process conditions. The commonly available tools as sequential chemical extraction and SEM-EDS analysis tend to underestimate the contribution of the metal sulphide precipitation. The influent-

effluent comparison of the sulphur species suggested that the amount of produced sulphide was capable of scavenging the metals in the system. Further experiments and additional methods should be used to explain these phenomena.

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EFFICACITE DES ZONES HUMIDES CONSTRUITES POUR LE POLISSAGE DES EAUX USEES EN PROVENANCE DE L'INDUSTRIE DE LA GALVANOPLASTIE

Spécialité: Sciences et génie de l'environnement
Mots clefs: cyanure, métaux, zones humides construites, polissage, traitement,

Résumé :

Les industries de galvanoplastie génèrent un volume important d'eaux usées qui contiennent des quantités élevées de métaux et de composés cyanurés. Ainsi, ces eaux contaminées peuvent représenter un danger pour l'environnement, si elles ne sont pas traitées correctement. A l'heure actuelle, l'utilisation de zones humides artificielles pour le traitement des eaux issues de la galvanoplastie a été très peu étudiée. Il est donc encore difficile de conclure quant à l'application à grande échelle de cette méthode en tant qu'étape de polissage en aval des systèmes de traitement conventionnels. En fait, les concentrations élevées de polluants toxiques dans les effluents de la galvanoplastie empêchent l'application directe des méthodes de traitement biologique. Ainsi, les zones humides artificielles ne sont pas une alternative aux méthodes physico-chimiques classiques de traitement des eaux usées. Cependant, après un traitement conventionnel des eaux usées, les zones humides artificielles pourraient représenter des méthodes de post-traitement peu onéreuses avant un rejet dans l'environnement; elles pourraient aussi constituer un volume tampon en cas de défaillance des installations de traitement amont.

Les objectifs de ce travail sont triples: (i) étudier la faisabilité du polissage des effluents de la galvanoplastie, (ii) étudier l'influence des systèmes opérationnels, des plantes, du substrat et de la charge des eaux usées, (iii) comprendre les mécanismes conduisant à une diminution de la charge métallique dans des microcosmes de zones humides construites.

L'expérience a été réalisée en utilisant deux systèmes expérimentaux composé de 12 colonnes dans chaque système. Le principal objectif du système expérimental mis en place est de faciliter une précipation des métaux (Cu, Ni, Pb, Zn) sous forme de sulfures, précipitation médiée par des bactéries réductrices de sulfate, qui, en général, sont anaérobies strictes. Ainsi, la plupart des colonnes ont été conçues pour être strictement anaérobies (8 sur 12 colonnes). Les conditions anaérobies dans les colonnes ont été permises par leur construction et leur mode de fonctionnement. Ces colonnes ont été exploitées dans un mode saturé à courant ascendant, en maintenant une couche d'eau de 6,5 cm en surface, afin d'empêcher l'air de pénétrer dans le substrat. Ces colonnes ont été alimentées par une pompe péristaltique à partir du fond, la sortie étant située 70 cm au-dessus. Cependant, les processus aérobies ont été également rapportés dans la littérature comme étant efficaces pour l'élimination des métaux et des cyanures. Par conséquent, des colonnes du système expérimental ont été conduites de façon intermittente en anaérobie et aérobie (4 sur 12 colonnes), en réalisant des cycles de remplissage et vidange en alternance. Les systèmes ont été alimentés soit avec des eaux usées de galvanoplastie, soit avec des eaux contaminées artificiellement par Al, B, Cu, Ni, Fe, Pb, Zn, des cyanures et des sulfates, éléments représentatifs des contaminants les plus préoccupants.

Cette thèse présente divers aspects du polissage des eaux usées de galvanoplastie par des zones humides artificielles : analyse de la performance, étude de la configuration et de la mise en œuvre, influence de différentes sources de carbone, évolution du substrat, l'analyse des plantes et des problèmes de conception.

Ce travail a fourni de nouvelles informations et élargi les connaissances actuelles sur le traitement des eaux usées industrielles dans les zones humides artificielles. En fait, il s'agit, à notre connaissance, de la première étude concernant l'élimination des métaux et du cyanure d'eaux usées par des zones humides artificielles en écoulement de sub-surface.

Les expériences ont été réalisées en utilisant deux systèmes expérimentaux, diffèrents par de nombreux points; une comparaisons inter-expérimental exhaustive ne peut donc être réalisée. En fait, dans une perspective de développement à grande échelle, il est essentiel d'étudier les performances du système envisagé dans un modèle de micro ou mésocosmes, sur place, en utilisant des eaux usées réelles. L'un des défis pour le polissage des eaux usées issues de la galvanoplastie est qu'elles contiennent de faibles concentrations résiduelles de métaux et sont très pauvres en matière organique. Cette particularité a été observée dans les deux systèmes et également lorsque les deux systèmes ont été comparés les uns aux autres.

Une élimination très efficace a été obtenue pour tous les métaux étudiés (Al, Cu, Ni, Zn,) sauf pour le Pb, en fonction de la qualité de l'effluent et la de configuration de la zone humide construite. D'un point de vue opérationnel seules les colonnes de flux ascendant méritent d'être considérées pour une application à grande échelle. De fait, les colonnes à cycles de remplissage et vidange alternatifs sont plus erratiques en exploitation et ont une forte propension au colmatage lorsque des substrats organiques sont utilisés.

L'élimination du cyanure est plus efficace dans les zones humides construites avec des conditions constantes d'anoxie que dans celles où les conditions d'oxydation et de réduction alternent.

Le bore ne peut être éliminé que lorsqu'une source de carbone interne, telle que du compost, est ajoutée. Cet élément est par contre conservé lorsque des substrats minéraux sont utilisés.

Des flux d'alimentation hydrique plus élevés permettent une meilleure efficacité d'élimination de métaux dans les colonnes à flux ascendant. Au contraire, pour les colonnes à remplissage et vidange alternatives un temps de rétention plus long dans les colonnes permet un retrait plus efficace des métaux.

L'élimination des métaux et des sulfates peut être augmentée lorsque du lactate est ajouté à l'effluent comme source de carbone externe, afin de stimuler l'activité des bactéries réductrices de sulfate. L'utilisation de lactate permet une efficacité de traitement plus élevée qu'en présence de la seule source de carbone interne et conduit à des concentrations comparables en matière organique dans l'effluent.

Les systèmes à remplissage et vidange alternatifs pourraient être utilisés comme 'réservoirs tampon d'urgence' pour prévenir la pollution de l'environnement par les eaux usées de galvanoplastie brutes en cas de défaillance de la station de traitement principale. Dans cette situation, les zones humides construites peuvent éliminer certains contaminants métalliques, diminuer la concentration en cyanures et diminuer significativement le pH très élevé des eaux usées. Cependant, le rejet des eaux usées de galvanoplastie, très agressives, peut exercer un effet défavorable important sur le rendement ultérieur de la zone humide artificielle, dû à certains contaminants.

Le rôle des plantes dans l'élimination des métaux, de bore et des cyanures est mineur. En outre, l'effet de la présence de la végétation sur l'efficacité du traitement est différent selon les contaminants métalliques; il est donc difficile de tirer des conclusions définitives sur ce point. Il

convient de souligner que les plantes limitent toutefois la prolifération des algues et offrent des avantages esthétiques à la zone humide construite.

Le processus attendu pour l'élimination des métaux était la précipitation des métaux avec du sulfure d'origine biogénique issu de l'activité des bactéries réductrices de sulfate. Ce processus ne joue finalement pas un rôle majeur dans l'élimination des métaux. La plus grande partie des métaux est liée à des fractions échangeables ou réductibles. Nous pouvons donc conclure que les processus d'adsorption et d'élimination aérobies sont responsables de l'élimination des métaux dans une expérience de courte durée. Ces processus ne sont pas recommandés parce que les métaux pourraient être facilement lessivés de ces phases à l'occasion de changement des conditions environementales. Les outils classiques d'extraction chimique séquentielle et d'analyse SEM-EDS ont tendance à sous-estimer la contribution de la précipitation des sulfures métalliques. La comparaison influent/effluent des formes du soufre suggère que la quantité de sulfure produite est capable de piéger les métaux dans le système. D'autres expériences et des méthodes supplémentaires devront être utilisées pour expliquer ces phénomènes.